IMPACT OF STABILIZED UREA FERTILIZERS ON GASEOUS NITROGEN LOSSES DURING FORAGE SEED PRODUCTION IN SASKATCHEWAN

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By

Nils Konstantin Ernst Yannikos

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ABSTRACT

Forage seed production requires significant fertility inputs, and differs from forage feed production in that fertilizer strategies focus on seed rather than biomass yield. Nitrogen fertilizer management is a particular challenge, because perennial grasses vary in their response to N due to differences in flower induction. Because forages grasses are typically grown for three or more years, N fertilizer typically is broadcast into standing vegetation.

Unfortunately, the surface application of urea—the most commonly used form of N fertilizer in Western Canada—is subject to a variety of losses, such as volatilization of ammonia (NH_3) and gaseous emissions of nitrous oxide (N_2O), resulting in a decrease in N use efficiency (NUE) and causing a risk for the environment. Furthermore, if fertilizers are applied in the fall, subsequent spring snowmelt can promote N_2O losses. One promising method to reduce these losses is to use stabilized fertilizers. Stabilized fertilizers contain either a urease or a nitrification inhibitor, or a combination of both, thereby blocking key pathways in the N cycle involved in NH_3 volatilization and N_2O emissions. The performance of stabilized fertilizers in soils of the Boreal Transition Zone, particularly under forage seed production management, is not well understood.

The performance of stabilized N fertilizers in reducing gaseous N losses was investigated by quantifying and comparing gaseous NH_3 and N_2O losses in forage seed production systems. A novel and cost-effective closed, dynamic flux chamber (CDFC) system for measuring NH_3 emissions in remote field sites was developed and validated.

Utilizing the CDFC system, a field study was conducted to assess the efficacy of surface-applied stabilized urea fertilizers in reducing gaseous NH_3 and N_2O losses from forage seed production sites after application either in fall or spring. The study identified application timing (i.e., fall *vs.* spring) as a dominant factor governing the magnitude of gaseous N losses, with the majority of NH_3 losses occurring after spring application, whereas N_2O losses were greatest from fall-applied fertilizers during spring snowmelt. Soil properties influenced the potential for gaseous N losses, and stabilized fertilizers containing urease inhibitors reduced NH_3 emissions significantly when the loss potential was high. The effect of stabilized fertilizers on N_2O emissions, on the other hand, varied strongly between field sites.

Soils were collected from the field sites and used in a series of bench-scale experiments to assess the efficacy of stabilized urea fertilizers in reducing NH_3 losses under different soil environmental conditions (i.e., soil pH, moisture, and temperature). The study identified strong differences between the NH_3 loss potential of the soils. Enhanced urea hydrolysis rates coupled with lower soil water content were the dominant factor governing the magnitude of NH_3 losses. Stabilized fertilizers containing both urease and nitrification inhibitors were most effective in reducing NH_3 losses.

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2-NPT	N-(2-nitrophenyl) phosphoric triamide
3-MP	3-methylpyrazole
AA	Anhydrous ammonia
ABC	Ammonium bicarbonate
ABR	Arborfield
AMO	Ammonia monooxygenase
AN	Ammonium nitrate
ANOVA	Analysis of variance
AUC	Area under the curve
BD	Bulk density
CAN	Calcium ammonium nitrate
CDFC	Closed, dynamic flux chamber
CEC	Cation exchange capacity
CHL	Choiceland
CR	Carrot River
DCD	Dicyandiamide
DMPP	3,4-dimethylpyrazole phosphate
DOY	Day of the year
EPA	Environmental Protection Agency
GWP	Global warming potential
HAO	Hydroxylamine oxidoreductase

HSD Honestly significant difference

IFA International Fertilizer Industry Association

LD Long day length induction

LD_{50} Lethal dose, 50%

MDCD Minimal detectable concentration difference

NBPT N-(n-butyl) phosphoric triamide

NBTPT N-(n-butyl) thiophosphoric triamide

NMDS Non-metric multidimensional scaling

NPTPT N-(n-propyl) thiophosphoric triamide

NUE Nitrogen use efficiency

OC Organic carbon

OM Organic matter

PPDA Phenyl phosphorodiamidate

RCBD Randomized complete block design

SD Short day length induction

SMA Saskatchewan Ministry of Agriculture

SOM Soil organic matter

SWE Snow water equivalent

TAN Total ammoniacal nitrogen

TDR Time domain reflectometry

TZ 1H-1,2,4-triazole

VMC Volumetric moisture content

1 INTRODUCTION

1.1 General introduction

Forage seed production requires significant fertility inputs, and differs from forage feed production in that fertilizer strategies should focus on seed rather than biomass production. Fertilizer management for this purpose requires a different approach than that for forage feed production, because for some perennial grasses, reproductive tillers are formed in fall (Heide, 1994). The Saskatchewan Ministry of Agriculture (SMA) recommends fall application of nitrogen (N), particularly for those grasses requiring vernalization of the reproductive tillers (Malhi et al., 2008). Because forage grasses are perennial and typically are grown for three or more years, N fertilizers cannot be incorporated into the soil, and therefore must be broadcast. In the past, ammonium nitrate (AN) was used for broadcast application as recommended by the SMA guidelines, because this fertilizer type quickly dissolves in water and moves into the soil where it can be rapidly taken up by the crop. Since AN is no longer readily available, broadcast application of urea, a more cost effective and widely available N fertilizer, has become the most common practice. Unfortunately, N in broadcast urea is subject to a variety of losses, such as gaseous emissions as ammonia (NH_3) via volatilization and as nitrous oxide (N_2O) via denitrification. These losses can reach a magnitude of more than 50% of applied N (Sommer et al., 2004), drastically reducing the nitrogen use efficiency (NUE) of the crops while releasing N_2O, a greenhouse gas of approximately 298 times the global warming potential of carbon dioxide (CO_2) (Myhre et al., 2014). Furthermore, the presence of plant residues—a situation especially common in perennial forage seed stands—has been shown to be one possible reason for increased urease activities, resulting in an enhanced hydrolysis of urea and thus increasing the potential for NH_3 losses through volatilization (Rochette et al., 2009a).

In contrast to conventional cropping systems, the production of forage seeds in the Canadian Prairies faces the challenge of supplying N to the crop when risks of losses are especially elevated, i.e., when N is applied in the fall and subsequently lost through nitrification/denitrification pathways during snowmelt, or when N is applied in the spring and lack of precipitation favors losses through NH_3 volatilization. Therefore, forage seed producers are looking for strategies to reduce

N losses from broadcast applications. Recently, several studies have shown promising results describing the use of urease and nitrification inhibitors to reduce volatilization and nitrification/denitrification losses in conventional cropping and animal grazing systems (Di and Cameron, 2002; Zaman et al., 2008; Zaman et al., 2009; Rochette et al., 2009b; Dawar et al., 2010). It remains to be seen whether the environmental benefits associated with the use of urease and nitrification inhibitors can be successfully transferred to forage seed production systems.

1.2 Research objectives

The main purpose of this study was to assess whether urease and nitrification inhibitors can successfully be used in forage seed production systems to reduce gaseous N losses as NH_3 and N_2O. For this purpose, a new system for measuring NH_3 emissions in the field was developed and a field study was conducted on existing forage seed production sites, where gaseous N losses were measured after surface application of stabilized fertilizers containing urease and nitrification inhibitors. Furthermore, soils from the field were brought to the lab and tested for their potential NH_3 emissions from stabilized fertilizers under different soil environmental conditions. The specific objectives of this study were to:

- develop and validate a system for measuring NH_3 emissions in the field that allows for a large number of treatment replications on remote sites, while being economically feasible;
- assess whether the surface application of stabilized urea fertilizers containing urease and/or nitrification inhibitors can reduce gaseous N emissions as NH_3 and N_2O relative to untreated urea; and
- assess the amount of NH_3 lost after surface application of stabilized fertilizers under controlled soil environmental conditions, such as soil moisture content, soil temperature, and soil pH.

1.3 Organization of the Dissertation

The research presented in this dissertation is organized in manuscript format. Chapter 2 presents a review of the literature highlighting the importance of gaseous N losses from urea on global nitrogen use efficiency (NUE) and the contamination of the environment, and the use of stabilized urea fertilizers to counter these problems. Chapter 3 presents the design and validation of a closed, dynamic flux chamber (CDFC) system suitable for measuring NH_3 emissions in remote field sites. Utilizing the CDFC design, Chapter 4 presents a field study assessing the efficacy of stabilized urea fertilizers in reducing gaseous N losses from forage seed production sites. The soils

from the field study are further assessed in Chapter 5, which examined the efficacy of stabilized urea fertilizers under different soil and environmental conditions. Chapter 6 synthesizes the results from all research chapters and closes with a conclusion.

2 LITERATURE REVIEW

2.1 Global importance of urea as a nitrogen fertilizer

Urea is the world's most consumed synthetic nitrogen (N) fertilizer (Sommer et al., 2004; Glibert et al., 2006; IFA, 2014) and since 2001 the consumption of urea alone exceeds that of all other mineral N fertilizers (Fig. 2.1). It represents the main growth sector in the N fertilizer industry and its demand is expected to grow further as global N demand increases (Heffer and Prud'homme, 2014). Developing countries have been the biggest consumers of urea fertilizers since the 1970's, and during that time, urea consumption in developing countries has increased by more than 1000%, whereas in developed countries it has only doubled (IFA, 2014). In 2012, South- and East Asia alone accounted for 86% of the world's urea consumption (IFA, 2014).

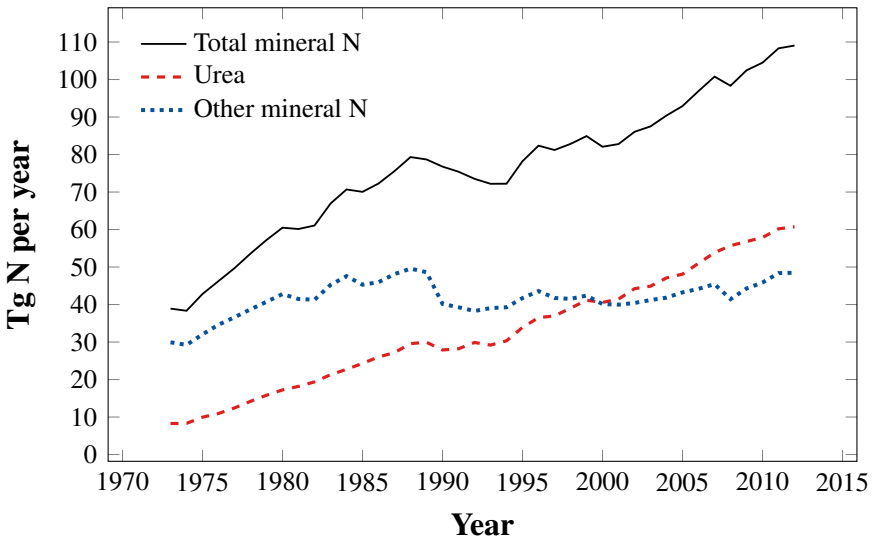


Fig. 2.1. Global consumption of mineral N fertilizers, adapted from IFA (2014).

In Western Canada, urea is the commonly used form of N fertilizer for agricultural production (Glibert et al., 2006), and 91% of urea consumed in Canada was shipped to Alberta, Saskatchewan, and Manitoba from July 2014 to June 2015 (Statistics Canada, 2015). Among all straight N fertil-

izers consumed in the Canadian Prairies, urea is used the most (68%), followed by urea ammonium nitrate (21%) and NH_3 (11%), whereas the amounts of ammonium nitrate are negligible (Statistics Canada, 2015). Urea is a N fertilizer, and although it is classified as an organic amide (and is also known as carbamide), it is often considered to be a mineral fertilizer, because it produces NH_4^+ upon hydrolysis.

Urea is produced by the reaction of anhydrous ammonia with carbon dioxide under high temperature and pressure (Glibert et al., 2006). Urea is less explosive than other fertilizers such as anhydrous ammonia or ammonium nitrate, and can therefore be stored and transported more easily. The N-content of urea is relatively high (46-0-0), therefore reducing the cost of labor during application to the field when compared to other fertilizer sources such as ammonium sulfate (21-0-0-24) (Glibert et al., 2006).

The use of urea as a fertilizer is associated with a variety of N losses from the soil/plant system (Kissel et al., 1977; Sommer et al., 2004; Trenkel, 2010), which reduce the N-use efficiency (NUE) and cause a negative impact on the environment through acidification and eutrophication of ecosystems (Schulze et al., 1989). The fertilizer industry has responded by developing new fertilizer products that reduce these losses. Trenkel (2010) classified these products into three categories: foliar fertilizers (i.e., fertilizers applied directly to the leaf surface of the crop); slow- and controlled-release fertilizers (i.e., fertilizers coated with permeable polymers that reduce the diffusion rate of urea from the granule); and stabilized fertilizers (i.e., fertilizers containing either a urease- and/or a nitrification inhibitor). While the use of foliar fertilizers is limited through leaf burn and N-uptake rates of the plants (Trenkel, 2010), the use of controlled release and stabilized fertilizers has shown most promising results in reducing N losses (Trenkel, 2010; Cameron et al., 2013).

2.2 Fertilizer management for forage seed production

Perennial forage grasses such as timothy (*Phleum pratense* L.) or bromegrass (*Bromus spp.* L.) have different induction requirements for flowering to produce seeds. Whereas the specific requirements can vary between perennial grass species, the three types of induction requirements that generally occur in forage grasses are: (a) cold temperature induction (vernalization); (b) short day length induction (SD); and (c) long day length induction (LD) (Heide, 1994). Many forage grasses, including bromegrass, require dual induction for flowering, which means that they need vernalization and/or SD to initiate inflorescence primordia (primary induction), as well as LD to initiate culm elongation, development of the inflorescence, and flowering (secondary induction) (Heide, 1994). Other forage grasses such as timothy, on the other hand, only require single induction, usually LD, in order to initiate flowering (Heide 1994). When application of N fertilizer

for seed production is considered, this physiological distinction between single and dual induced forage grasses becomes an important factor affecting the efficient timing of fertilizer application. For dual induced forage grasses, N fertilizer should be applied in the fall before before the onset of primary induction, thus supporting the development of reproductive tillers suitable for seed production in the coming year. For single induced forage grasses, on the other hand, N fertilizers should be applied before LD induction, which usually occurs in early spring.

As a result, the timing of fertilizer application (i.e., fall or spring) can strongly affect the yield performance of perennial forage grasses, depending on the species-specific requirements of the crop. Furthermore, because forage grasses are perennial and are typically grown for three or more years, N fertilizers cannot be incorporated into the soil and thus need to be broadcast. The broadcast application of urea, the most commonly used N fertilizer in forage seed production, is prone to NH_3 volatilization losses (Sommer et al., 2004). Moreover, the application of fertilizer N in the fall, as required for dual induced forage grasses, exposes the applied N to losses via nitrate leaching and gaseous losses of nitrous oxide (N_2O) during spring snowmelt. New products such as controlled release and stabilized fertilizers are therefore promising candidates to reduce the N losses associated with forage seed production.

2.3 Processes by which gaseous N losses from urea occur

2.3.1 Hydrolysis of urea

The main difference between urea and other forms of N fertilizers, such as ammonium bicarbonate (ABC), ammonium nitrate (AN), anhydrous ammonia (AA), and calcium-ammonium-nitrate (CAN) is that urea needs to be hydrolyzed in order to release NH_4^+. Moreover, N losses, such as NH_3 volatilization, NO_3^- leaching, and N_2O emissions can only occur after urea has been hydrolyzed, therefore making urea hydrolysis one of the key processes involved in N losses from the soil system (Sommer et al., 2004; Saggar et al., 2013b).

Urea is hydrolyzed by urease, an enzyme common in most soils, and the widespread presence of this enzyme—urease is produced by bacteria, algae, fungi, and plants—is likely related to the high abundance of urea across ecosystems (Sommer et al., 2004; Krajewska, 2009; Saggar et al., 2013b). Soil urease is an extracellular enzyme that is derived from microbes and dead plant cells, and its adsorption to clay and humic particles prevents decomposition, thereby resulting in the accumulation of urease within soils (Krajewska, 2009).

Urease catalyzes the hydrolysis of urea to carbamic acid and NH_3 (Krajewska, 2009), and carbamic acid then further hydrolyzes to carbonic acid and NH_3 (Fig. 2.2)(Kiss and Simihăian, 2002; Sommer et al., 2004; Krajewska, 2009; Saggar et al., 2013b). The reaction increases the pH in

the immediate reaction environment (Kiss and Simihăian, 2002; Sommer et al., 2004; Krajewska, 2009; Saggar et al., 2013b).

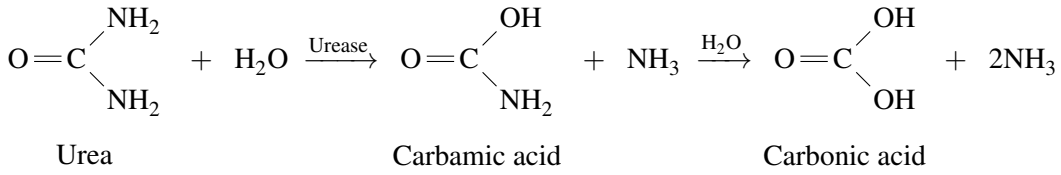


Fig. 2.2. Chemical pathway of the hydrolysis of urea to carbonic acid and NH_3, adapted from Krajewska (2009).

The rate of hydrolysis by urease follows Michaelis-Menten kinetics (Cabrera et al., 1991; Krajewska, 2009) and increases with temperature when moisture is not limiting (Vlek and Carter, 1983; Sommer et al., 2004). At high urea concentrations, however, the hydrolysis by urease is inhibited (Krajewska, 2009). The rate of urea hydrolysis in soils has been shown to be strongly correlated with soil organic carbon and nitrogen (Dick, 1984) and the cation exchange capacity and clay content of the soil (Dharmakeerthi and Thenabadu, 1996). Generally, the hydrolysis of applied urea after application to soils is complete within 10 d at 5°C and within 2 d at 30°C (Trenkel, 2010).

Although the pH optimum for urease has been reported to lie between pH 7 and 8 (Krajewska, 2009) or between pH 8 and 9 (Sommer et al., 2004), soils can vary in urease activity, irrespective of soil pH (Sommer et al., 2004). Moreover, because the hydrolysis reaction is dependent on water (Fig. 2.2), it can be inhibited when soil moisture content is low (Sommer et al., 2004).

A common way of assessing the potential of a soil to rapidly release NH_3 is to measure urease activity in soils. This is usually done using enzyme assays, during which a known concentration of urea is applied to the soil and the released ammoniacal N (i.e., NH_4^+/NH_3) is measured colorimetrically after incubation (e.g., at 37°C) for a duration of 30 to 120 min (Tabatabai and Bremner, 1972; Nannipieri et al., 1978; Kandeler and Gerber, 1988; Klose and Tabatabai, 1999).

Although the hydrolysis of urea is an important step for making urea-N available to plants, it can favor NH_3 volatilization losses from surface applied urea, particularly if no precipitation occurs to move the urea into the soil (Sommer et al., 2004). In this case, the hydrolysis of urea causes NH_3 to be released at the soil surface, where it is susceptible to volatilization losses.

2.3.2 Ammonia volatilization

Ammonia volatilization is the result of diffusive transport of NH_3 from the soil surface to the laminar atmospheric layer at the soil-air interface, followed by turbulent transport away from the

source above the laminar layer (Sommer et al., 2004). The rate of NH_3 emissions is dependent on the transfer coefficient—which is highly dependent on wind speed—and the concentration gradient of NH_3 between the soil-air interface and the free atmosphere, as shown in Eq. 2.1 (Sommer et al., 2004):

$$F_v = K_b \times (\chi - \mathrm{NH_{3,a}}) \qquad \text{(Eq. 2.1)}$$

where F_v is the emission (flux) rate of NH_3, K_b is the transfer coefficient, χ is the partial pressure of NH_3 at the soil-air interface, and $\mathrm{NH_{3,a}}$ is the partial pressure of NH_3 in the free atmosphere.

The concentration of gaseous NH_3 at the soil-air interface is dependent on the concentration of NH_3 in solution and the temperature, as shown in Eq. 2.2 (Sommer et al., 2004):

$$\chi = \mathrm{NH_{3,L}} \times 10^{1477.6/T - 1.69} \qquad \text{(Eq. 2.2)}$$

where χ is the partial pressure of NH_3 at the soil-air interface, $\mathrm{NH_{3,L}}$ is the concentration of NH_3 in the soil solution, and T is the temperature (K).

High concentrations of NH_3 in the soil solution can therefore cause significant volatilization losses, and this effect is stronger when wind turbulence removes diffused NH_3 from the atmospheric layer above the soil surface. On the other hand, the absence of air movement above the soil surface—conditions that occur during placement of static measurement chambers—can reduce NH_3 volatilization losses, because the accumulation of NH_3 above the soil surface lowers the concentration gradient between soil solution and the atmosphere (Sommer et al., 2004).

The concentration of NH_3 in solution can be described as a function of total ammoniacal nitrogen (TAN), pH, and temperature, as shown in Eq. 2.3 (Sherlock and Goh, 1984; Sommer et al., 2004):

$$\mathrm{NH_{3,L}} = \frac{\mathrm{TAN}}{1 + 10^{(0.09018 \,+\, 2729.92 \,/\, \mathrm{T} \,-\, \mathrm{pH})}} \qquad \text{(Eq. 2.3)}$$

where $\mathrm{NH_{3,L}}$ is the NH_3 concentration in solution (mol L^{-1}); TAN is the total ammoniacal N concentration (mol L^{-1}); T = temperature (K); and pH = pH of the solution.

This model predicts how the proportion of NH_3 in the TAN pool increases with temperature when pH is raised above 8, and how this effect intensifies with further increases in pH, whereas below a pH of 8, the majority of TAN will be in the NH_4^+ form, regardless of the temperature (Fig. 2.3).

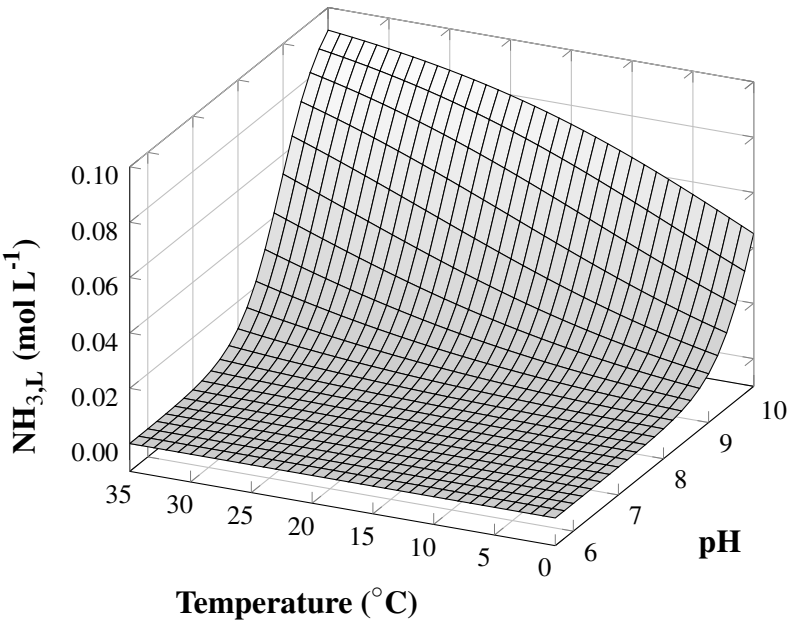


Fig. 2.3. Impact of pH and temperature on aqueous NH_3 concentration in the soil solution with a TAN concentration of 0.1 mol L^{-1}, according to Eq. 2.3 (Sherlock and Goh, 1984), adapted from Sommer et al. (2004).

Within the model, soil pH is the most important factor determining whether TAN is mostly present as NH_3 or as NH_4^+. The application of urea to soils low in pH causes TAN to be transformed to NH_4^+, which can be adsorbed to soil colloids and is not susceptible to NH_3 losses (Sommer et al., 2004). Because the reaction product of urea hydrolysis (i.e., NH_3, see Fig. 2.2) is a base with $pK_b = 4.76$, it increases soil pH in the reaction environment (Sommer et al., 2004). When urea is banded near the soil surface, the high concentration of urea in bands can cause a strong increase in pH upon hydrolysis, shifting TAN towards NH_3 and potentially resulting in higher NH_3 losses than from surface applied urea (Sommer et al., 2004; Rochette et al., 2009b). Furthermore, because urea granules are hygroscopic, high air humidity can favor hydrolysis of surface-applied urea, even when the soil is dry, resulting in high concentrations of NH_3 at the soil surface followed by volatilization losses (Black et al., 1987b; Sommer et al., 2004).

2.3.3 Nitrous oxide emissions

Another important N loss pathway from urea is through emissions of nitrous oxide (N_2O). Nitrous oxide is a key greenhouse gas with the global warming potential (GWP) 298 times that of CO_2 over a 100-year time scale (Myhre et al., 2014). Nitrous oxide is produced in soils predominantly through microbially mediated nitrification and denitrification processes (Braker and

Conrad, 2011; Cameron et al., 2013). Terrestrial soils are assumed to be responsible for 62% to 70% of global N_2O emissions, the remainder originating from oceans, power plants, and vehicles (Braker and Conrad, 2011; Cameron et al., 2013). The increase in the atmosphere has been attributed to increases in agricultural activity, as soil N_2O emissions increase with N availability as a direct response to N management (Rochette et al., 2008).

Nitrification is the transformation of NH_3 to NO_3^- under aerobic conditions in a two-step reaction (Braker and Conrad, 2011). The first step is the oxidation of NH_3 to hydroxylamine (NH_2OH) through the enzyme ammonia monooxygenase (AMO) (Fig. 2.4). Hydroxylamine is then further transformed to NO_2^- by the enzyme hydroxylamine oxidoreductase (HAO). During the second reaction step, NO_2^- is oxidized to NO_3^- (Braker and Conrad, 2011) (Fig. 2.4).

$$NH_3 + O_2 + 2H^+ + 2e^- \xrightarrow{\text{AMO}} NH_2OH + H_2O \xrightarrow{\text{HAO}} NO_2^- + 5H^+ + 4e^-$$

$$NO_2^- + H_2O \rightarrow NO_3^- + 2H^+ + 2e^-$$

Fig. 2.4. Chemical pathway of nitrification (Braker and Conrad, 2011). AMO = ammonia monooxygenase, HAO = hydroxylamine oxidoreductase.

Nitrous oxide is formed during ammonia oxidation through chemical decomposition of hydroxylamine (Braker and Conrad, 2011), and although the formation of NO_2^- occurs at rates several orders of magnitude higher than that of N_2O, the high radiative forcing of N_2O makes this loss pathway an environmental concern. Furthermore, the end product of the nitrification process (i.e., NO_3^-) is a precursor for denitrification.

Denitrification is the reduction of NO_3^- to N_2 through the intermediates NO_2^-, NO, and N_2O, as shown in Fig. 2.5 (Braker and Conrad, 2011). Moreover, denitrification requires anaerobic conditions and is generally the largest source of N_2O emissions from soils, accounting for losses of up to 30% of applied fertilizer N (Braker and Conrad, 2011). In contrast to N_2O as a facultative intermediate during nitrification, its formation during denitrification is obligate. Furthermore, the lack of the gene *nosZ* in most microbial communities—the gene responsible for the reduction of N_2O to N_2—results in N_2O often being released as the main product of denitrification (Braker and Conrad, 2011).

$$NO_3^- \xrightarrow{NO_3^- \text{ reductase}} NO_2^- \xrightarrow{NO_2^- \text{ reductase}} NO \xrightarrow{\text{NO reductase}} N_2O \xrightarrow{N_2O \text{ reductase}} N_2$$

Fig. 2.5. Stepwise reduction of NO_3^- to N_2 during denitrification (Braker and Conrad, 2011).

In most soils, nitrification and denitrification occur simultaneously due to differences in soil aeration conditions between soil microsites (Fowler et al., 2009; Braker and Conrad, 2011), with net N_2O emissions as the product of both processes. Braker and Conrad (2011) explain the spatial and temporal variability in N_2O emissions as the result of biogeochemical "hot spots" and "hot moments" for denitrification. According to McClain et al. (2003), a biogeochemical denitrification hot spot or hot moment is an area or time window in which denitrification rates are strongly increased compared to the surrounding area or the majority of the time.

2.4 Use of stabilized N fertilizers to mitigate N losses

One of the most promising methods of mitigating NH_3 and N_2O losses is the use of stabilized fertilizers (Trenkel, 2010; Cameron et al., 2013). Stabilized fertilizers block key processes in the N cycle, such as urea hydrolysis and/or nitrification, through the addition of urease and/or nitrification inhibitors, respectively.

2.4.1 Urease inhibitors

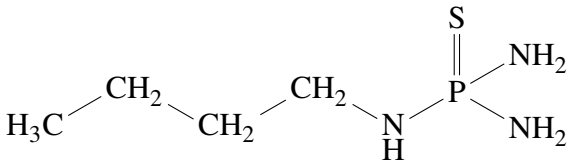
Urease inhibitors prevent or delay the hydrolysis of urea to ammoniacal N (Fig. 2.2) by inhibiting the activity of the enzyme urease. When applied together with urea, urease inhibitors preserve applied N in the form of urea for 7 to14 d, thus reducing NH_3 volatilization losses. This provides farmers with more flexibility in terms of expanding the N fertilizer application timing window. Furthermore, the delayed hydrolysis of urea lowers the impact of NH_3 toxicity to seedlings when applied in bands (Trenkel, 2010).

Among thousands of tested compounds suitable for inhibiting the enzyme activity of urease, only a few compound groups were considered for agricultural use, mainly due to their non-toxicity, high efficiency at low concentrations, and degradability in the soil (Trenkel, 2010). Organophosphoric structural analogues of urea are considered the compound group of largest agricultural importance globally (Trenkel, 2010; Saggar et al., 2013b). Phenyl phosphorodiamidate (PPDA) (Fig. 2.6) was one of the first compounds of this group that was assessed for agricultural use (Liao and Raines, 1985; Pedrazzini et al., 1987). N-(n-butyl) thiophosphoric triamide (NBTPT[1]) (Fig. 2.6) was later shown to be more efficient than PPDA (Bremner and Chai, 1989), and has received extensive attention since 1990 (Saggar et al., 2013b). To date, NBTPT represents one of the most intensively studied and distributed urease inhibitors (Kiss and Simihăian, 2002; Saggar et al.,

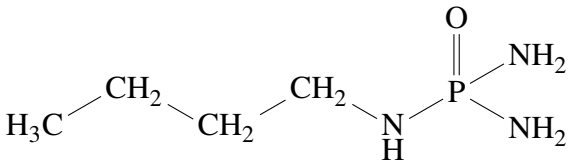
[1] In the literature, N-(n-butyl) thiophosphoric triamide is often abbreviated as NBPT, therefore causing confusion in the distinction between its thiophosphoric (NBTPT) and phosphoric (NBPT) form. To avoid confusion when abbreviating these components, the approach by Christianson et al. (1993), Krajewska (2009), and Saggar et al. (2013b) is followed throughout this dissertation, and the two compounds are abbreviated accordingly as NBTPT and NBPT.

2013b). It has been marketed under the trade name Agrotain® since 1996 (Trenkel, 2010) and currently is being distributed by Koch Agronomic Services, LLC, USA. Recently, BASF released a urease inhibitor formulation under the trade name Limus®, consisting of a mixture of the active ingredients NBTPT and N-(n-propyl) thiophosphoric triamide (NPTPT) (Li et al., 2015). The use of NBTPT is risk-free for the environment, and the non-toxicity of NBTPT—the oral LD_{50} was reported at 1000 to 4000 mg kg^{-1}—makes this compound safe to handle (Trenkel, 2010). Another more recent member of the group of the organo-phosphoric compounds is N-(2-nitrophenyl) phosphoric triamide (2-NPT) (Fig. 2.6), which was patented in 2005 and is currently under development for marketing of a stabilized urea fertilizer under the trade name Piazur® by SKW Stickstoffwerke Piesteritz GmbH, Germany (Hucke et al., 2005).

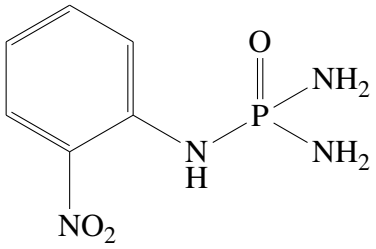
Thiophosphates differ from phosphates only in that a sulfur atom instead of an oxygen atom is bound to the phosphorus atom (Fig. 2.6). Besides the structure of the organic substituent (i.e., -butyl *vs.* -nitrophenyl groups), this represents the main difference between NBTPT and 2-NPT (Kiss and Simihăian, 2002; Saggar et al., 2013b). Indeed, NBTPT needs to be transformed to its oxygen analogue N-(n-butyl) phosphoric triamide (NBPT) before it can effectively inhibit urea hydrolysis (Phongpan et al., 1995; Saggar et al., 2013b); the actual inhibition of urea hydrolysis is therefore attributed to the inhibitory effect of phosphoric amides (i.e., in NBPT and 2-NPT) on the active site of the urease enzyme (Trenkel, 2010; Saggar et al., 2013b).



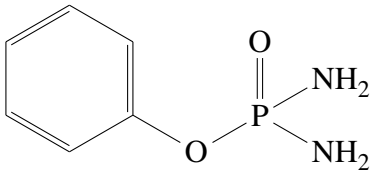
N-(n-butyl) thiophosphoric triamide
(NBTPT)



N-(n-butyl) phosphoric triamide
(NBPT)



N-(2-nitrophenyl) phosphoric triamide
(2-NPT)



Phenyl phosphorodiamidate
(PPDA)

Fig. 2.6. Chemical structure of the urease inhibitors N-(n-butyl) thiophosphoric triamide (NBTPT), its oxygen analogue N-(n-butyl) phosphoric triamide (NBPT), N-(2-nitrophenyl) phosphoric triamide (2-NPT), and phenyl phosphorodiamidate (PPDA).

2.4.2 Nitrification inhibitors

Nitrification inhibitors depress the nitrification activity of *Nitrosomonas* by inhibiting the oxidation of NH_3 to hydroxylamine (Fig. 2.4) for four to ten weeks (Amberger, 1989; McCarty, 1999; Sommer et al., 2004; Trenkel, 2010; Cameron et al., 2013; Saggar et al., 2013b). This prevents the conversion of NH_3 to NO_3^- in the soil, therefore reducing the risk for NO_3^- leaching and N_2O emission losses. Many compounds have been assessed for their use as nitrification inhibitors, but only those with little or no impact on the environment, as well as sufficient economic and agronomic benefits, are currently in use (Trenkel, 2010).

Among the most popular nitrification inhibitors currently in use are dicyandiamide (DCD), 1H-1,2,4-triazole (TZ), 3-methylpyrazole (3-MP), 2-chloro-6-(trichloromethyl) pyridine (Nitrapyrin), and 3,4-dimethylpyrazole phosphate (DMPP) (Fig. 2.7) (Trenkel, 2010).

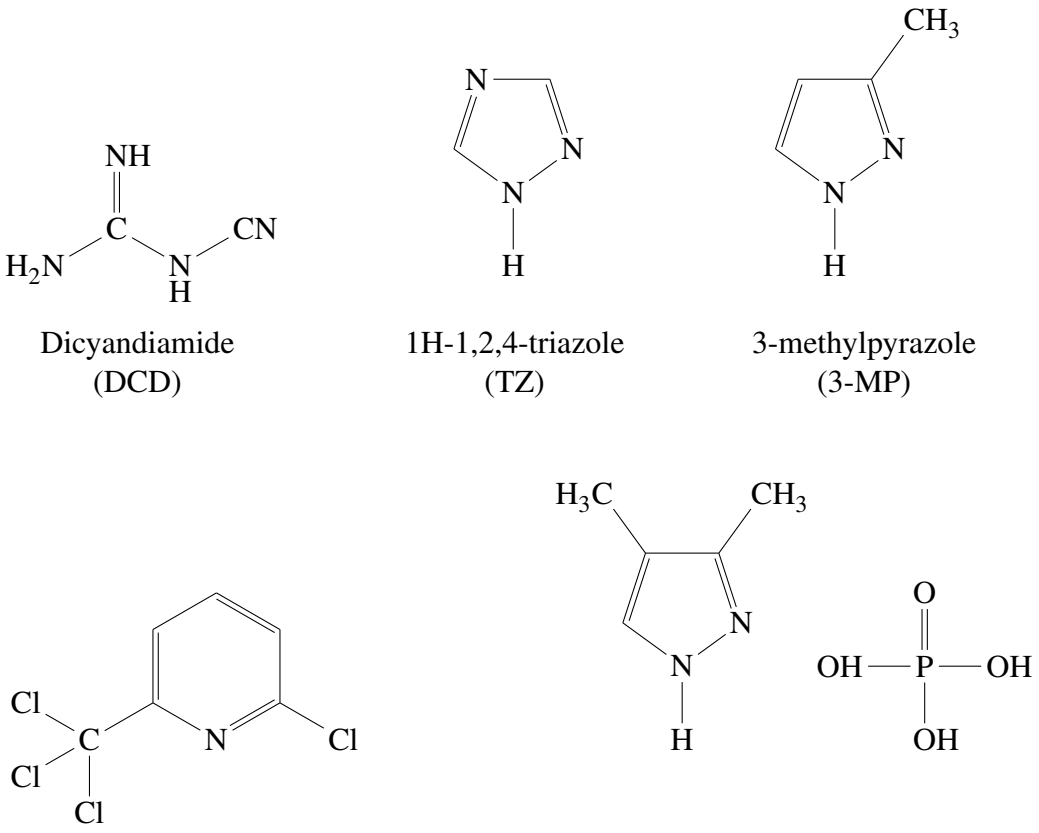


Fig. 2.7. Chemical structure of the commonly used nitrification inhibitors dicyandiamide (DCD), 1H-1,2,4-triazole (TZ), 3-methylpyrazole (3-MP), 2-chloro-6-(trichloromethyl) pyridine (Nitrapyrin), and 3,4-dimethylpyrazole phosphate (DMPP) .

Dicyandiamide is one of the most popular and longest-known nitrification inhibitors (Kelliher et al., 2008; Trenkel, 2010). It was tested for its use in agriculture as early as in 1917, although its use was originally intended as a N fertilizer rather than a nitrification inhibitor (Linter, 1917). Its development as a nitrification inhibitor dates back to the 1960s, and the main countries developing DCD were Germany and Japan (Amberger, 1989). In 1984, SKW Trostberg introduced DCD to the United States (Trenkel, 2010), and to date it is used in the commercial products Alzon® by SKW Stickstoffwerke Piesteritz GmbH, as well as in Agrotain® Plus and SuperU™ by Koch Agronomic Services, LLC (Trenkel, 2010). Dicyandiamide is a bacteriostatic reagent—but not bactericidal—that inhibits the enzyme ammonia monooxygenase and therefore prevents the formation of NO_2^-. It is considered non-toxic with a LD_{50} of 10,000 mg kg^{-1} (Amberger, 1989; Trenkel, 2010). In the presence of metal oxides, it decomposes part biologically and part chemically via guanylurea,

guanidine, and urea to NH_3, CO_2, and H_2O (Fig.2.8); thus it can also be considered a slow-release N fertilizer. To reduce the application rate of DCD without losing efficiency, it is often applied in combination with other nitrification inhibitors, such as 1H-1,2,4-triazole (e.g., as a mixture with DCD in Alzon®) and 3-methylpyrazole (Hucke et al., 2005; Trenkel, 2010).

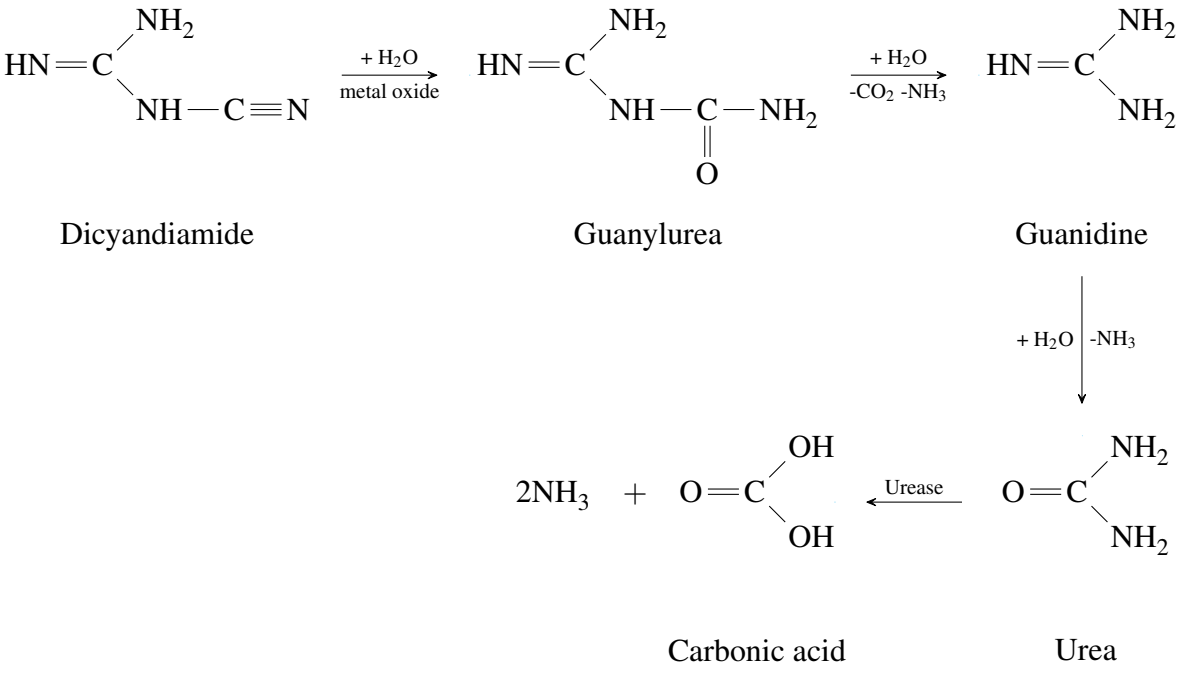


Fig. 2.8. Decomposition of dicyandiamide (DCD) in the soil to carbonic acid and NH_3, adapted from Amberger (1989).

Dicyandiamide alone has been used for reducing NO_3^- and N_2O losses from cattle urine patches in pasture soils in New Zealand (Di and Cameron, 2002, 2004a; Zaman et al., 2007, 2013). Until 2013 it was marketed under the product name Eco-N™ in New Zealand by Ravensdown, but was voluntarily withdrawn by the manufacturer in 2013 after traces of DCD were found in dairy products (Craymer, 2013). In Western Europe, instead of DCD, a mixture of TZ and 3-MP under the trade name Piadin® by SKW Stickstoffwerke Piesteritz GmbH is generally applied to slurries and grasslands (Trenkel, 2010).

Nitrapyrin is one of the first nitrification inhibitors that were accepted in the United States by the Environmental Protection Agency (EPA). It was registered in 1974 and is marketed under the trade name N-Serve® by Dow Agro Sciences. Nitrapyrin acts as a bactericide on *Nitrosomonas*, which results in a strong inhibitory effect on the oxidation of NH_3 while also killing part of the microbial population (Trenkel, 2010). The LD_{50} dose of nitrapyrin lies at 2,140 mg kg^{-1}, and in

soil and plants it is rapidly degraded into N, Cl, CO_2, and H_2O within 30 d of application (Trenkel, 2010).

3,4-Dimethylpyrazole phosphate (DMPP) is one of the most recently developed nitrification inhibitors. Developed by BASF in 1995, it is now distributed under the trade name Entec® (Trenkel, 2010). It is highly immobile, preventing it from leaching into the soil. Compared to nitrapyrin, it does not have bactericidal effects, although it reduced the abundance of ammonia oxidizing bacteria in a long-term study (Trenkel, 2010; Ruser and Schulz, 2015). Furthermore, it requires a lower dose than DCD to effectively inhibit nitrification. Its LD_{50} dose lies between 300 and 2,000 mg kg^{-1} and leaves no residues in plants upon decomposition (Trenkel, 2010).

2.4.3 Efficacy of urease and nitrification inhibitors in reducing N losses

Urease and nitrification inhibitors, alone or in combination, have been used successfully to mitigate N losses. The majority of the studies focus on their use in either grazed dairy systems, crop production, or application to slurry and manure (e.g., Di and Cameron, 2004a,b; Engel et al., 2011; Singh et al., 2013; and Li et al., 2015). Typically, regardless of land-use system, urease inhibitors are able to reduce NH_3 losses compared to untreated urea (e.g., Sanz-Cobena et al., 2008; Zaman et al., 2008; Engel et al., 2011; and Ni et al., 2014). Reductions in NH_3 losses by urease inhibitors are significant in most studies, but the magnitude of the reducion is dependent on NH_3 loss rate from unamended urea, N application rate, soil moisture content, pH and OM (Watson et al., 1994; Saggar et al., 2013b), and soil temperature (Carmona et al., 1990). As a result, the reduction in NH_3 volatilization losses by urease inhibitors is highly variable. For example, Li et al. (2015) observed a reduction in NH_3 losses from surface-applied granular urea treated with a mixture of the urease inhibitors NBTPT and NPTPT (Limus®) ranging from 76% to 100% of applied N in a winter wheat crop. In other examples, reductions in NH_3 losses from granular surface-applied urea treated with urease inhibitors were reported as 90% (Watson et al., 1994), 42% (Sanz-Cobena et al., 2008), 45% (Zaman et al., 2008), 89.5% (Turner et al., 2010), 66% (Engel et al., 2011), 58% (Abalos et al., 2012), 47% (Singh et al., 2013), and 26 to 83% (Ni et al., 2014). Similar results were observed when the urease inhibitor NBTPT was added as a liquid to cow urine and subsequently applied in a grazed pasture, reducing NH_3 losses by 29 to 93% (Zaman et al., 2009). Engel et al. (2013) demonstrated that NBTPT was more efficient in inhibiting urea hydrolysis in alkaline soils as a result of rapid degradation of NBTPT under acidic conditions. Furthermore, the inhibition of urea hydrolysis was enhanced at lower temperatures (i.e., 0.5°C *vs.* 20°C) as a result of slowed decomposition (Engel et al., 2013).

Despite the efficiency of urease inhibitors in reducing NH_3 volatilization losses observed in most studies, the plant N uptake or grain yield often is not significantly increased (McKenzie et al., 2010; Abalos et al., 2012; Singh et al., 2013; Li et al., 2015).

Nitrification inhibitors often are used when climatic conditions and soil management regime favor extensive NO_3^- leaching and N_2O emission losses. Grazed pasture systems in temperate climates are especially susceptible to these losses, because concentrations of N within urine patches can be as high as 1000 kg N ha^{-1}, exceeding the plant uptake capacity and potentially causing environmental pollution through NO_3^- leaching, NH_3 volatilization, and N_2O emissions (Saggar et al., 2013b). Extensive research has been conducted on DCD in reducing these losses from grazed dairy pasture systems in New Zealand, where the majority of NO_3^- and N_2O losses originate from cattle urine (Di and Cameron, 2002; Luo et al., 2010; Saggar et al., 2013b). When applied as solution or fine particle suspension at a rate of 7.5 to 15 kg ha^{-1}, dicyandiamide was successful in inhibiting nitrification of urea-N originated from urine patches, reducing NO_3^- leaching by 61% to 76% and N_2O emissions by 16% to 82% (Di and Cameron, 2002, 2003, 2004a,b, 2005, 2006; Kelliher et al., 2008; Luo et al., 2010; Zaman et al., 2013). Dicyandiamdide was more effective in autumn than during the summer, because the decomposition of DCD is slowed at lower temperatures (Di and Cameron, 2004a; Kelliher et al., 2008). The application of nitrification inhibitors resulted in different effects on NH_3 emissions when compared with untreated urea or urine, ranging from no effect (Di and Cameron, 2004a; Ni et al., 2014) to increased NH_3 losses (Zaman et al., 2009, 2013). In a meta study assessing the impact of nitrification inhibitors on NH_3 volatilization losses, Kim et al. (2012) reported contradicting results; out of 46 data sets analyzed, 26 stated an increase in NH_3 losses, while 14 resulted in no change, and 6 studies reported a decrease in NH_3 losses. Regardless of land use or fertilizer type, soils high in pH and low in CEC showed the strongest increase in NH_3 volatilization losses from nitrification inhibitors (Kim et al., 2012).

Urease inhibitors are often applied in combination with nitrification inhibitors (i.e., double inhibitor) to minimize effects on both NH_3 volatilization and N_2O emissions. Double inhibitors generally reduce total N losses (i.e., NH_3-N, NO_3^--N, and N_2O-N) (Zaman et al., 2009; Luo et al., 2010; Zaman et al., 2013), although in some instances, the presence of a nitrification inhibitor increases NH_3 losses when applied in combination with a urease inhibitor (Zaman et al., 2008). When NBTPT was applied together with DCD, Zaman et al. (2013) observed an increased decomposition of NBTPT due to acidity formed by the dissolution together with DCD and single superphosphate. Furthermore, Singh (2007) reported that nitrification inhibitors alone reduced emissions of N_2O more strongly than a combination of both inhibitors. Luo et al. (2010) suggested that the result for the reduced efficiency of double inhibitors is based on two processes: (a) the decomposition of the nitrification inhibitor while the urease inhibitor preserves N in the urea form, resulting in increased N_2O emissions relative to fertilizers that contain a nitrification inhibitor only; and (b) the presence of the nitrification inhibitor preserves N in the NH_3/NH_4^+ form, potentially increasing NH_3 losses.

2.5 The role of stabilized N fertilizers in forage seed production

The majority of management methods for reducing N losses from urea are focused on minimizing N losses, such as NH_3 volatilization, NO_3^- leaching, and N_2O emissions. According to the 4R concept for fertilizer management (Fertilizer Canada, 2016), the N use efficiency can be optimized if fertilizer N is applied using (i) the right source, (ii) the right application rate, (iii) the right time, and the (iv) the right place.

Surface applications of urea play an important role in forage seed production and are generally prone to NH_3 volatilization losses as a result of increased concentrations of ammoniacal N at the soil surface (Sommer et al., 2004; Cameron et al., 2013; Saggar et al., 2013b). One of the most common management procedures to mitigate these losses is therefore the incorporation of urea into the soil (i.e., the right place), usually to a depth of 3 to 5 cm (Sommer et al., 2004; Krajewska, 2009; Frame et al., 2012; Cameron et al., 2013; Saggar et al., 2013b). This places the N source away from the soil surface and allows NH_4^+ to be diluted in the soil solution and adsorbed to soil colloids before reaching the soil surface (Sommer et al., 2004). However, as the hydrolysis of urea raises the soil pH within the urea bands (Sommer et al., 2004; Krajewska, 2009; Rochette et al., 2009b), in dry acidic soils, this effect can increase NH_3 volatilization losses compared to surface-applied urea (Rochette et al., 2009b). Stabilized urea fertilizers have the potential to reduce N losses from surface application, thereby reducing the need for incorporation of the fertilizer. Furthermore, if stabilized urea fertilizers are incorporated, the delayed hydrolysis of urea can expand the time window for the urea to move into the soil, thereby limiting the increase in soil pH and thus reducing the potential for NH_3 losses.

The timing of N application is another important management factor for forage seed production, because forage grasses vary in their fertilizer requirement. Application timing can affect the potential losses of N through NH_3 volatilization or N_2O emissions. When fall application of N is required, this increases the risk for losses via N_2O emissions and NO_3^- leaching during spring snowmelt. Spring application, on the other hand, may increase the risk for NH_3 losses as temperatures increase. Furthermore, because urea hydrolysis is dependent on the availability of water (Fig. 2.2), the soil moisture status can affect the potential for NH_3 losses. Application of urea to a dry soil can reduce the risk of NH_3 losses due to the reduced activity of urease under dry conditions. Application to a moist soil, on the other hand, increases the risk for NH_3 losses, because urea will be hydrolyzed upon contact with the soil (Sommer et al., 2004). Moreover, urea applied to the surface of a dry soil can result in significant losses when air humidity is high, because urea granules will absorb moisture and become hydrolyzed. Applying urea before precipitation is therefore an important control measure for limiting NH_3 volatilization losses, because urea is subject to convective transport into the soil (Sommer et al., 2004). Black et al. (1987a) demonstrated that NH_3 losses could be reduced by 80% after 10 to16 mm of artificial precipitation was applied. Sim-

ilar results were found by Engel et al. (2011), who found that precipitation events of more than 18 mm reduced volatilization losses to less than 10% of applied N. Generally, precipitation events of more than 20 mm are considered to reduce NH_3 losses completely (Sommer et al., 2004).

Using stabilized N fertilizers for forage seed production in Saskatchewan can reduce the risk for N_2O emissions from fall application by blocking nitrification, thereby reducing the concentration of NO_3^- available for denitrification during spring snowmelt. If applied in the spring, stabilized fertilizers can reduce the potential for NH_3 losses by delaying the hydrolysis of urea. Furthermore, stabilized fertilizers protect the applied N from premature urea hydrolysis and NH_3 emissions when applied to moist soil, thus expanding the application timing window.

3 Design and Validation of a Closed Dynamic Flux Chamber System to Measure Ammonia Emissions Under Field Conditions

3.1 Preface

To assess whether stabilized fertilizers can successfully reduce gaseous NH_3 losses in the Boreal Transition Zone of Saskatchewan, it is important to monitor these losses under field conditions. However, many measurement systems are costly and require in-field power (e.g., wind tunnels) or large field sites (e.g., micrometeorological methods), thereby making it difficult to facilitate a sufficient number of treatment replicates required for testing the effect of stabilized fertilizers on NH_3 losses. Consequently, in this study a cost-effective closed, dynamic flux chamber was developed and validated under field conditions.

3.2 Abstract

Ammonia (NH_3) volatilization from mineral fertilizers is one of the most important N loss pathways from cropping systems (Sommer et al., 2004). However, assessing the magnitude of NH_3 losses in the field is challenging, in part because some of the factors that affect NH_3 volatilization (e.g., wind speed and soil temperature) can be affected by the measurement systems (Pacholski et al., 2006). Ammonia emissions can be measured using a variety of systems, including micrometeorological and chamber-based systems—the latter including both dynamic and static chambers. In general, the impact of these systems on the factors influencing NH_3 emissions decreases with increasing complexity of the system. For example, micrometeorological systems are among the most accurate measurement systems, but are also among the most complex systems and require a large relatively homogeneous field area, which make it unsuitable for comparative studies involving large numbers of replicated treatments, or for the use in remote locations (Pacholski et al., 2006).

Another commonly used measurement system involves the use of small wind tunnels (Lockyer, 1984) that minimize the invasive effect of the tunnel on factors governing NH_3 emissions, and are more suitable for replicated studies. However, wind tunnel systems are generally costly and require a permanent setup; consequently, their use is generally limited. In Saskatchewan, many crop and forage production systems are potentially significant contributors of NH_3 emissions; however, few studies have attempted to measure *in situ* NH_3 losses in the field. The aim of this study was to develop and validate a cost-effective, chamber-based system for measuring NH_3 emissions at remote field sites.

When the system was validated in the field using three urea application rates (i.e., 0, 46, and 92 kg N ha^{-1}), it was capable of detecting significant treatment differences using sampling times as short as 30 min even though cumulative losses over a 10-d period were low (i.e., 1.1% of applied urea-N). A sampling time of 90 min was recommended to ensure establishment of constant atmospheric conditions within the chamber. When the system was validated in the lab, recovery efficiencies increased with increasing exposure time. It was concluded that the system was useful for detecting differences in NH_3 emission patterns and treatment-induced differences in NH_3 emissions, especially at remote sites and in multi-treatment studies.

3.3 Introduction

Volatilization of ammonia (NH_3) is one of the major N-loss pathways associated with broadcast applications of urea fertilizer—a commonly used and cost-efficient N source. The loss of N reduces the N-use efficiency (NUE) of the fertilizer and poses an environmental threat as a result of off-site deposition and subsequent acidification and eutrophication of ecosystems (Schulze et al., 1989; Sommer and Hutchings, 1995; Asman et al., 1998; Sommer et al., 2004). Thus, accurate measurement of NH_3 volatilization is an important prerequisite for developing and testing management strategies to mitigate N losses.

The most common methods for measuring NH_3 emissions in the field involve the absorption of NH_3 in an acid medium (followed by laboratory analysis of the acid solution) or direct detection of the NH_3 using infrared absorption spectroscopy (McGinn and Janzen, 1998). However, there are a number of difficulties associated with the measurement of NH_3; e.g., NH_3 is a "sticky" gas that easily adsorbs to certain materials, especially copper and stainless steel tubing (McGinn and Janzen, 1998). Moreover, because NH_3 readily dissolves in water, condensation within the tubing or on the walls of the chamber can lead to an underestimation of volatilized NH_3 (McGinn and Janzen, 1998). Environmental factors such as wind speed, precipitation and temperature affect the rate of NH_3 volatilization (Sommer et al., 2004; Harper, 2005); therefore, consideration of these

factors plays an important role in the design and functionality of any system used to measure NH_3 emissions.

There are a variety of measurement methods available to detect NH_3 emissions under field conditions (Harper, 2005; Miola et al., 2015), and these differ significantly in both their complexity and the degree to which they disturb the soil microclimate. Harper (2005) categorized NH_3 measurement systems as those that interfered with the transport process (e.g., enclosure-based methods) and those that minimized this interference (e.g., micrometeorological methods). As the name implies, noninterfering methods—such as Eddy covariance methods—do not alter the environmental conditions (e.g., wind speed, precipitation/irrigation, radiation and soil temperature, and partial pressure of NH_3 at the soil/air interface) under which the NH_3 emissions occur. They are generally preferred when field-scale emission factors are to be assessed and are considered the most reliable systems for measuring NH_3 emissions associated with the application of animal slurry or mineral fertilizers (Sommer et al., 2004; Harper, 2005; Pacholski et al., 2006; Gericke et al., 2011). Moreover, these systems allow for near-continuous measurement while minimizing the effects of localized variations in NH_3 emissions from the soil by averaging the measurement over a large area (Harper, 2005). Due to low atmospheric concentrations of NH_3 above the field, however, micrometeorological measurements require sensors that are highly sensitive and have a very rapid response time (Harper, 2005)—and which are generally quite expensive. The most important drawback of micrometeorological methods is that they need to be situated in large and homogeneous fields that need to be surrounded by unfertilized land, thus making them unsuitable for replicated measurements in agronomic multi-plot field experiments (Sommer et al., 2004; Harper, 2005; Pacholski et al., 2006; Gericke et al., 2011)

Enclosure methods, on the other hand, are commonly used in agronomic experiments where relative differences in NH_3 emissions from experimental plots are to be evaluated. Enclosure methods involve covering a proportion of soil with a chamber or wind tunnel and capturing gaseous NH_3 within the enclosure during a predetermined time period. The benefits of enclosure methods lie in their simplicity, sensitivity and portability (Sommer et al., 2004). Covering the soil with an enclosure, however, interferes with factors governing NH_3 volatilization by altering the temperature and wind speed within the chamber, and providing surfaces for water condensation and NH_3 absorption. Thus, enclosure methods often tend to over- or underestimate NH_3 volatilization rates, thus yielding an estimate of the NH_3 emission potential rather than the actual flux under undisturbed field conditions (Sommer et al., 2004; Harper, 2005; Pacholski et al., 2006). On the other hand, enclosure methods allow for the comparison of different fertilizer or animal slurry treatments in replicated, multi-plot field experiments (Pacholski et al., 2006; Miola et al., 2015).

There are many different enclosure systems available for detecting NH_3 emissions, and can vary in size (i.e., the area of soil covered), air speed within the chamber, and the method by which

the NH_3 is captured (i.e., active *vs.* passive collection). Static chambers are used to measure the increase in NH_3 within the chamber during the time that the chamber is closed. A passive sampler, usually an acid-soaked filter or an open bottle containing an acid solution (e.g., 0.01 M H_2SO_4), is placed inside the chamber to absorb gaseous NH_3. Static chamber systems, however, can have a negative feedback on gaseous emissions of NH_3 within the enclosure, because the absence of air exchange can result in lower emission rates than would occur outside the chambers, therefore resulting in underestimation of gaseous NH_3 losses (Sommer et al., 2004).

Dynamic chamber systems differ from static chamber systems in that the NH_3-enriched air is usually drawn through the chamber and sampled either actively or passively (Sommer et al., 2004). Wind tunnels, such as the ones developed by Lockyer (1984), can cover areas up to 1 m^2 and function by placing an open Plexiglas tunnel over the soil and capturing the NH_3 in the air entering and leaving the chamber using automated samplers located at the air inlet and outlet of the tunnel. The flux is then calculated based on the difference in NH_3 concentration at the air outlet of the wind tunnel (relative to the concentration at the air inlet), the surface area enclosed by the wind tunnel, and the air flow through the tunnel. Wind tunnels do not alter the wind speed and are therefore regarded as the enclosure system with the greatest accuracy. The presence of the wind tunnel, however, can alter soil moisture by sheltering the soil during precipitation events. And though this can be avoided by removing the wind tunnel during such events, this can be quite laborious and requires permanent weather monitoring. Wind tunnels also require a reliable supply of in-field power which, together with the high cost associated with these systems, limits their utility—especially in remote locations.

Smaller dynamic chamber systems, such as the ones developed by Kissel et al. (1977), usually utilize fans and/or vacuum pumps to increase the wind speed within the chamber and pump the NH_3-enriched air through an acid trap. Similar systems have been developed for the use in both field and laboratory settings (Zaman et al., 2008; Rochette et al., 2009b; Zaman et al., 2009; Woodward et al., 2011; Miola et al., 2015). The air flow through a dynamic chamber is maintained at a relatively high speed to facilitate the transfer of NH_3 from the soil to the atmosphere (thus mimicking the real-world situation); consequently, the measured emissions are generally greater than those obtained using a static chamber (Sommer et al., 2004). Although dynamic chamber systems for measuring NH_3 emissions have been in use for more than 40 years, the rapid development of open-source computer-controlled router tables during the past decade has drastically reduced the cost of constructing individual chambers (Pearce et al., 2010). This, combined with the production of low-cost miniaturized pumps and improved battery performance has made it possible to deploy dynamic chamber systems more widely and in more remote locations than was previously possible. The aim of this study was to design, construct and validate a dynamic chamber system for the measurement of NH_3 emissions at remote field locations.

3.4 Materials and Methods

3.4.1 System design

The chambers (Fig. 3.1) were constructed of clear acrylic, with a sheet thickness of 6.3 mm. All acrylic parts were professionally cut on a computer-controlled router table and were solvent-welded together. The outer dimensions of each chamber were 15.3-cm tall $\times$ 46.0-cm long $\times$ 22.0-cm wide, with the inner dimensions covering an area of 0.098 m^2. Acrylic square stocks (1.3-cm $\times$ 1.3-cm $\times$ 10.0-cm) were welded to the inside corners to reinforce the chambers and act as guides to set the depth of installation at 5.3 cm, resulting in an internal volume of 9.8 L after installation. The chamber lid consisted of an acrylic sheet (46.0-cm $\times$ 22.0-cm) with acrylic square stocks welded along each edge to stabilize the lid. Two small acrylic sheets (10.0-cm tall $\times$ 20.0-cm long) were welded to the underside of the lid to act as air-flow guides (Fig. 3.1f). A battery holder (Fig. 3.1d) was attached to the outward face of one of the air guides and an air-circulation fan was mounted between the guides (Fig. 3.1e). An air pump (Parker Hargraves CTS micro diaphragm pump; Model # A.1C19N1.C06VDC; Hargraves Technology Corporation; Mooresville, NC) was mounted on the underside of the lid and connected to the air outlet (Fig. 3.1g). To ensure a consistent air-flow and pump speed (2 L min^{-1}), the fan and the air pump were controlled using Pololu step-up/step-down voltage regulators (Model No. U3V12F5 and S7V8A, respectively; Pololu Corporation; Las Vegas, NV) (Fig. 3.2). The air intake was located at the other end of the lid (180° from the outlet). Foam rubber weather stripping (12.5-mm wide $\times$ 6.3-mm thick) was glued along the edge of the underside of the lid (Fig. 3.1k) to provide a tight seal between the lid and the chamber.

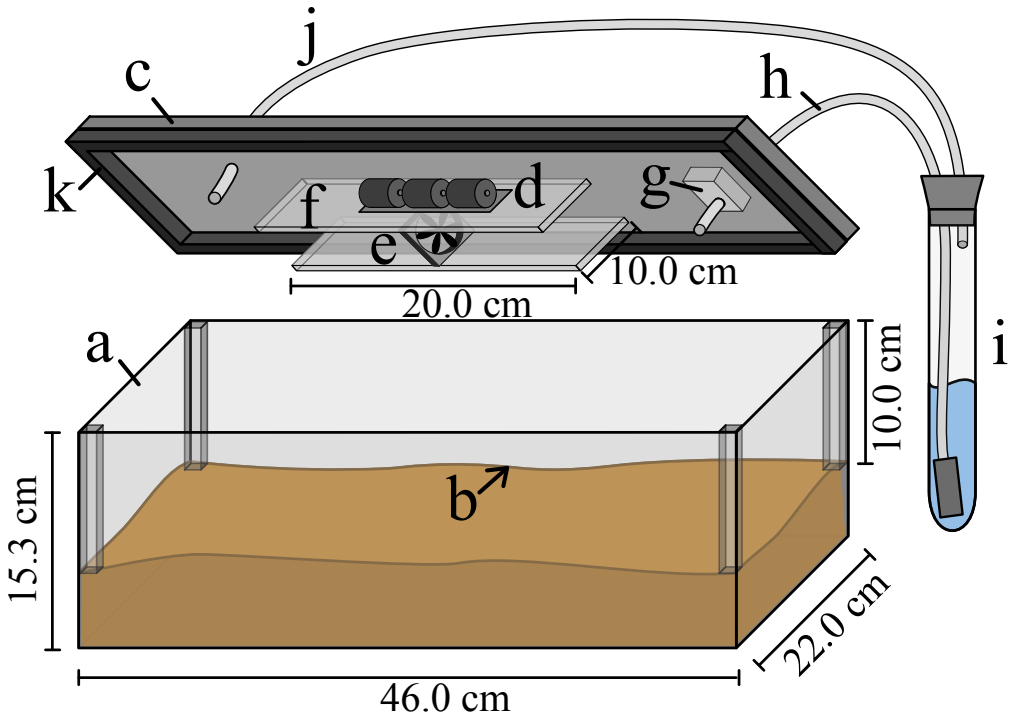


Fig. 3.1. Schematic of the closed, dynamic flux chamber (CDFC) system: The (a) base frame, (b) the soil surface, (c) the lid, (d) a battery holder containing three D-cell batteries, (e) a fan, (f) two air guide baffles, (g) an air pump, (h) a vinyl hose connected to the output of the pump, (i) an acid trap vial with a bubble stone, (j) a vinyl hose entering the chamber, and (k) a foam rubber weather stripping.

Upon deployment (Fig. 3.3), the pump is turned on and atmospheric air enters the chamber through the inlet, is guided across the soil surface, and then exits the chamber through the outlet. The fan and baffles mounted on the underside of the lid increase the air speed within the chamber and guide the air along the soil surface. The chamber is operated in this configuration for 10 min to ensure equilibration with the external atmosphere. The acid trap (35 mL 0.01 M H_2SO_4) is then attached between the air inlet and outlet (Fig. 3.1i) and NH_3-free air is circulated through the chamber for 90- to 360-min.

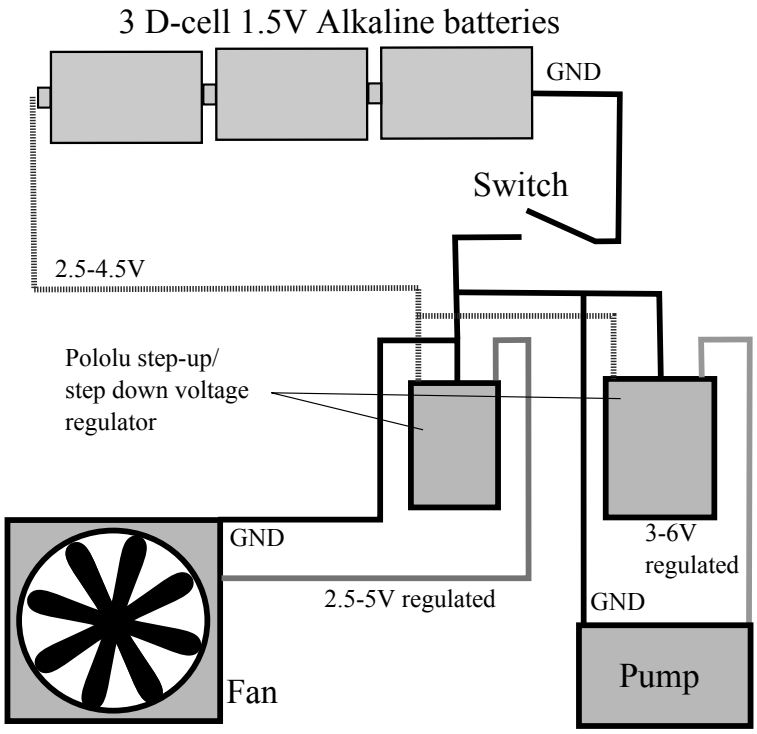


Fig. 3.2. Circuit plan of the chamber lid.

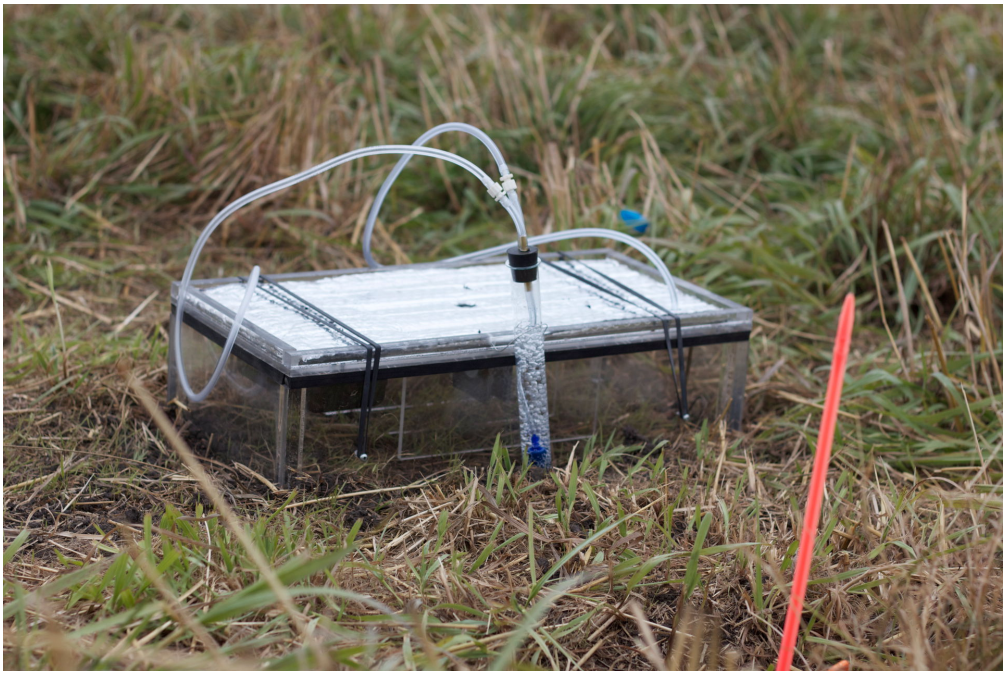


Fig. 3.3. Closed, dynamic flux chamber in the field while trapping gaseous NH_3 in acid.

3.4.2 System validation

System performance was assessed by placing an NH_3 source inside a closed chamber, operating the chamber under controlled conditions, and determining the amount of NH_3 recovered in the acid trap and in condensation on the exposed inner surfaces of the chamber. The standard flux chamber (Fig. 3.1) was modified by solvent-welding a piece of acrylic to the chamber base (Fig. 3.4). The acrylic base (44.7-cm long $\times$ 22.0-cm wide, Fig. 3.4c) was cut to fit inside the chamber and was attached to the inner walls of the chamber using the corner reinforcements as guides to set the depth; i.e., so that the modified chamber had the same "above-ground" volume (9.8 L) as in the field. A 100-mL glass beaker (Fig. 3.4a) was inserted into a custom-cut hole in the bottom plate and sealed in place with silicone, and the lower portion of the beaker wrapped with a heating strip (Fig. 3.4b).

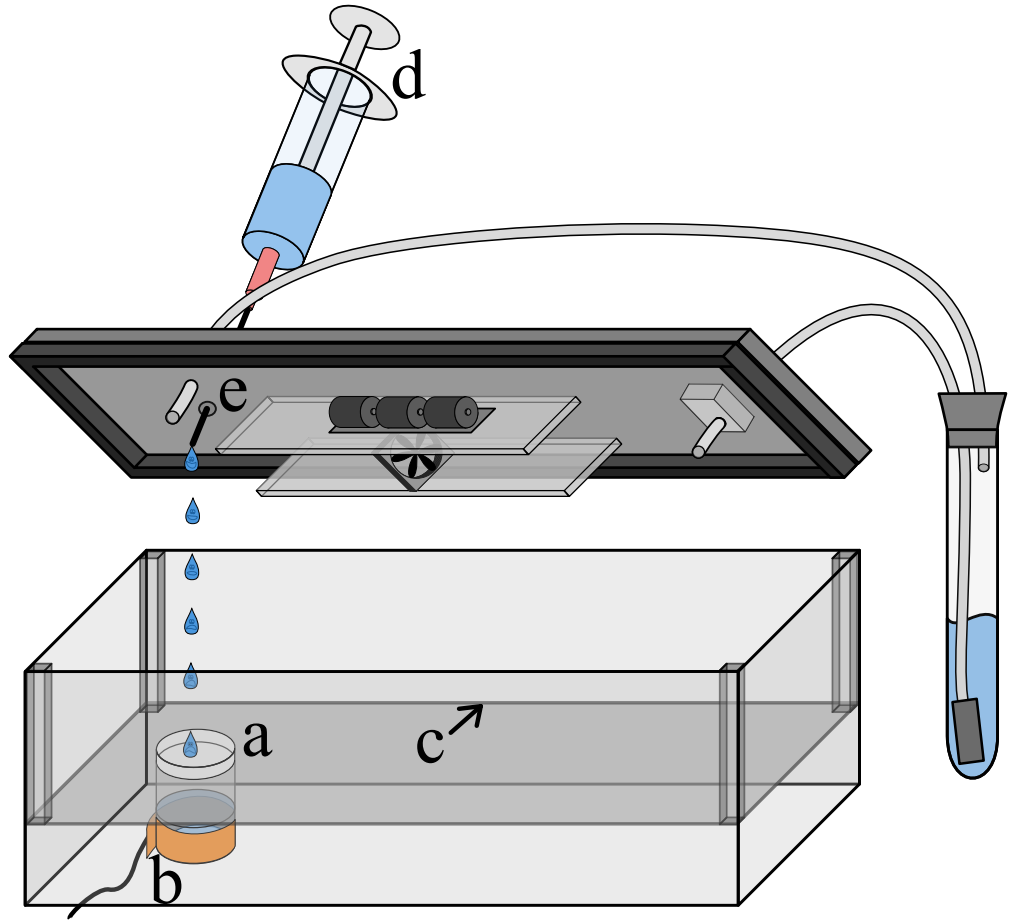


Fig. 3.4. Schematic of the modified chamber system for validation: The 100 mL beaker containing (a) the NH_4Cl solution, (b) the heating strip wrapped around the beaker, (c) the custom-cut Plexiglas plate, (d) the syringe applying either NaOH or H_2SO_4 through a rubber septum in the lid (e).

At the start of each test run, 40 mL of 0.0143 *M* NH_4Cl (i.e., 8.01 mg NH_4^+-N) were added to the beaker, the solution heated to 30°C, the chamber lid closed and the acid trap attached, and the pump started. A 20-mL aliquot of 0.5 *M* NaOH was then injected into the beaker through a sampling port in the lid (Fig. 3.4e). The addition of NaOH resulted in an increase in the solution pH to ca. 12, thus facilitating the conversion of NH_4^+ to NH_3. As well, volatilization was enhanced by heating the solution to 30°C. Thirty-five minutes after adding the NaOH, a 20-mL aliquot of 0.53 *M* H_2SO_4 was added to the solution and the heating tape was turned off. This step brought the total volume in the beaker to 80 mL and lowered the pH to ca. 2, essentially terminating the production of NH_3. The headspace air in the chamber was circulated through the acid trap (35 mL 0.01 *M* H_2SO_4) for 90, 150, 240, or 360 minutes (following addition of the NaOH). Each test run was replicated four times. At the end of test run, the pump and fan were switched off and the acid trap removed and analyzed for NH_4^+. The concentration of NH_4^+ in the acid traps was determined colorimetrically using a SmartChem® 200 Discrete Wet Chemistry Analyzer (Westco Scientific Instruments Inc.; Brookfield, CT, USA). Percentage recovery of the added NH_4^+ was then calculated using Equation 3.1:

$$RE = \frac{m_a}{m_i - m_f} \times 100 \qquad \text{(Eq. 3.1)}$$

where *RE* is the recovery efficiency (%); m_i = initial amount of NH_4^+ (8.01 mg N) in solution added to the chamber; m_f = amount of NH_4^+ (mg N) remaining in solution upon completion of the controlled volatilization; and m_a = amount of NH_4^+ (mg N) recovered in the acid trap. In addition to the acid traps, NH_3 absorbed in condensation water inside the chamber was recovered by wiping the interior surfaces with a Kimwipe™ dipped in 0.01 *M* H_2SO_4. The interior surfaces of the chamber were wiped dry and the paper towel placed in a volumetric flask. The volumetric flask was filled with 250-mL of 0.01 *M* H_2SO_4 and left over night to equilibrate. The solution was then analyzed for NH_4^+ as described above.

3.4.3 Field Experiment

An experiment was conducted to assess the performance of the chambers under field conditions. The experiment was set-up at the University of Saskatchewan Goodale Research Farm located approximately 10 km south of the city of Saskatoon, SK Canada. Soils at the site are mapped as Dark Brown Chernozems of the Bradwell Association and developed on medium to moderately coarse textured sandy glacio-lacustrine deposits (Rostad, 1979). Chamber bases (n = 12) were installed in a randomized complete block design with three fertilizer treatments, including an unamended control, replicated four times. The fertilizer treatments involved applications of granular urea at rates equivalent to 46 and 92 kg N ha^{-1} (i.e., 9.87 and 19.75 g urea m^{-2}, re-

spectively). Once the chamber bases were in place, 3 mm of water was surface applied to the soil enclosed by the chamber base to improve conditions for urea hydrolysis. For the fertilizer treatments, the granular urea was broadcast applied to the center of the chamber. A lid was then placed onto the base and secured using rubber bands (see Fig. 3.3); the pump and fan were turned on and the chambers were allowed to equilibrate with the external atmosphere for 10 minutes. After equilibration, an acid trap containing 35 mL of 0.01 M H_2SO_4 was connected to each chamber and the chamber air circulated through the trap for 30 min. At the end of the 30 min, the acid trap was replaced by a fresh trap containing 35 mL of 0.01 M H_2SO_4; this procedure was repeated at 30-min intervals for a total of 120 minutes. The chambers were sampled daily—at the same time of day (i.e., between 11:00 and 13:00)—for 10 d. Upon completion of the daily sampling, the acid traps were stored in a refrigerator at 4°C until they were analyzed. Ammonium concentrations in the trap solutions were determined as described in Section 3.4.2

3.4.4 Statistical analysis

All statistical analyses were performed using the open-source statistics program "R" (ver. 3.0.2) (R Foundation for Statistical Computing, 2014). Cumulative NH_3 emissions were calculated using the area-under-the-curve function in the "flux 0.3-0" package in R (Jurasinski et al., 2015), which assumes linearity in the NH_3 flux between sampling times. Normality of the data and variance homogeneity were tested using the Shapiro Wilk's test and Levene's test, respectively. A univariate ANOVA was conducted and significant differences were tested post-hoc using Tukey's HSD test.

3.5 Results and Discussion

3.5.1 System validation

Ammonia recovery ranged from 45% after a 90-min deployment to 96% after a 360-min deployment (Table 3.1). In general, the recovery efficiency increased as a linear function of the deployment time (Fig. 3.5). The general trend was according to our expectations, because a constant pump speed through the acid trap will decrease the NH_3 concentration within the chamber, ultimately requiring more than 360 minutes to completely remove NH_3 from the head space. Higher pump speeds have shown to increase the total loss of NH_3 from the soil solution (Kissel et al., 1977; San Francisco et al., 2011), due to a reduction in the partial pressure of NH_3 in the atmosphere (Sommer et al., 2004).

Table 3.1. Recovery of NH_3 during bench-scale performance tests of the closed dynamic flux chamber.

Time	m_i	m_f	Δm	m_a	RE
min	———	mg N	———		%
90	8.58	6.98	1.60	0.72	45.00
150	8.41	7.24	1.17	0.69	58.97
240	8.37	7.14	1.23	0.73	59.35
360	8.09	7.28	0.81	0.78	96.30

† m_i = initial amount of NH_4^+ in solution added to the chamber; m_f = amount of NH_4^+ remaining in solution upon completion of the controlled volatilization; $\Delta m = m_i - m_f$ = amount of NH_3 released from solution; m_a = amount of NH_4^+ recovered in the acid trap; and *RE* is the recovery efficiency (%).

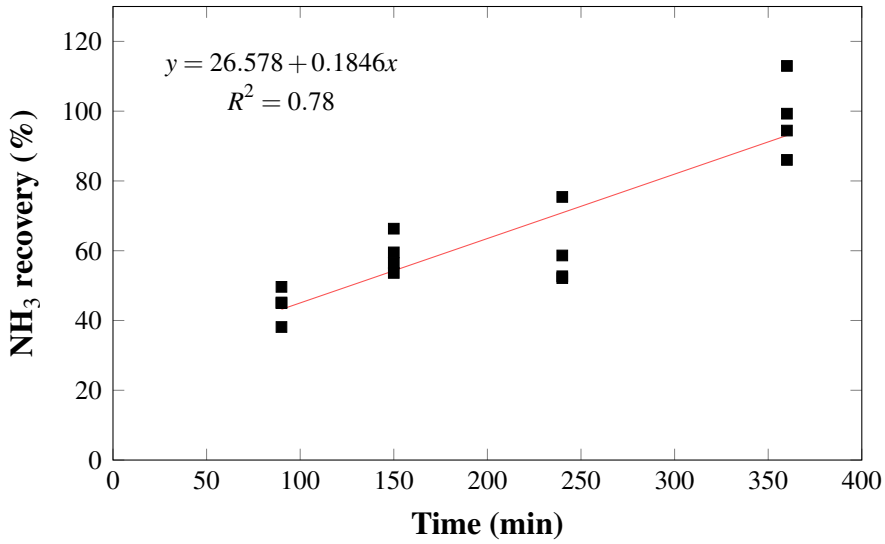


Fig. 3.5. Recovery of volatilized NH_3 as a function of time.

Interestingly, the total amount of NH_3 released from the NH_4Cl solution decreased with increasing deployment time (i.e., ranging from 18.6% with a 90-min deployment to 10.0% with a 360-min deployment; Table 3.1). This is likely a result of the acidification (to a pH of ca. 2) of the source solution at t = 35 min; i.e., the "source" solution was essentially converted into an acid trap that would then re-absorb some of the previously released $NH_{3(g)}$. Consequently, the near complete removal of gaseous NH_3 after a 360-min deployment is likely a result of re-absorption

of $NH_{3(g)}$ by the "internal trap" as opposed to an increase in recovery efficiency by the external trap, and it is likely that the true recovery efficiency lies below that. These data suggest that, at the current pump speed of 2 L min^{-1}, an increase in sampling time does not increase the amount of trapped NH_3 to a degree that would outweigh the added time requirements for sampling in the field. Thus, a relatively short deployment time (e.g., 90 min) may be suitable for field experiments where the focus lies on comparing treatment effects on NH_3 volatilization.

3.5.2 Field validation

Ammonia emissions were measured in the field over a 10 d period in July 2013. In general, NH_3 emissions from the unfertilized control soil were quite low (averaging 0.80 ± 1.33 μg N m^{-2} min^{-1}) and remained relatively unchanged during the 10 d measurement period (Fig. 3.6). Ammonia emissions increased with increasing application rate of the urea fertilizer, with significant emission events on DOY (Day of the year) 191 and DOY 195 (Fig. 3.6).

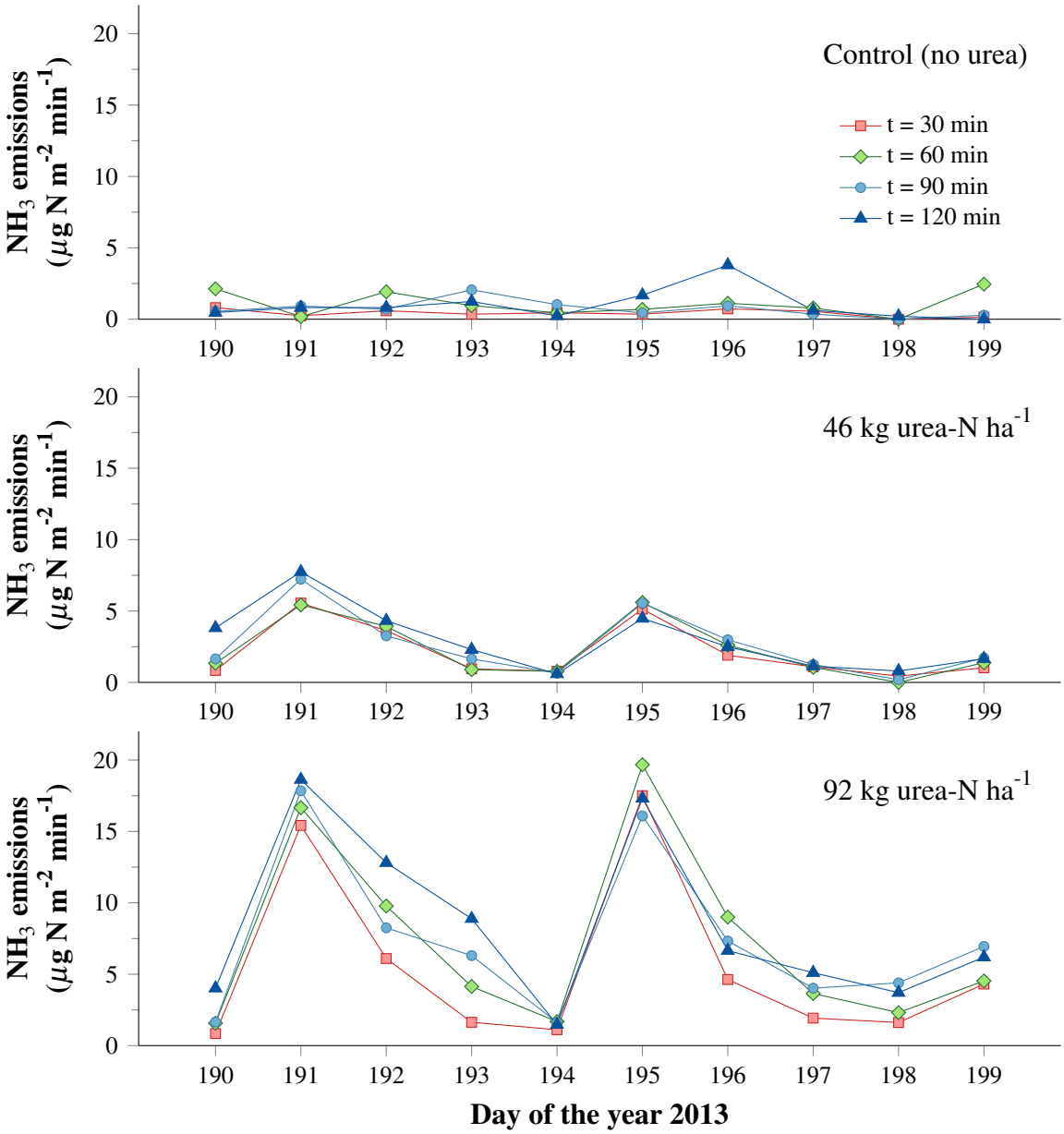


Fig. 3.6. Rates of NH_3 emission from field soils amended with surface-applied urea at three rates (i.e., 0, 46, 92 kg N ha^{-1}). Emission rates were measured from replicate (n = 4) chambers with the acid traps changed at 30-min intervals over a 2-h deployment period.

The initial emission event occurred 24-h after application of the urea fertilizer—together with approximately 3 mm of artificial precipitation. The second emission event occurred 4 d later following a small (5.4 mm) precipitation event. These results presumably reflect the fact that precipitation can induce hydrolysis of the urea granules at the soil surface, thus resulting in NH_3 volatilization (Sommer et al., 2004).

During chamber deployment, the NH_3 emission rate was generally lowest during the initial (0 to 30 min) sampling interval and greatest during the final (90 to 120 min) sampling interval. Indeed, NH_3 emission rates measured at 30 to 60, 60 to 90, and 90 to 120 min remained relatively constant (i.e., were not significantly different; $P = 0.254$). Moreover, when observed, significant differences between sampling intervals occurred only between the initial and final intervals. These data suggest that—following a short (ca. 30 min) establishment period—atmospheric transfer conditions within the chambers become relatively constant. Cumulative NH_3 emissions were estimated using an area-under-the-curve (AUC) analysis of the daily emission rate *vs.* time curves (see Fig. 3.6). Cumulative NH_3 emissions from the control plots (11.5 $\pm$ 2.4 mg N m^{-2}) were significantly ($P \leq 0.001$) lower than those from either of the urea amended plots (Table 3.2), but were within the range reported for soils in Quebec (Miola et al., 2015) and British Columbia (Bittman et al., 2005) over a similar timeframe (i.e., 7 to 14 d). In general, cumulative NH_3 emissions increased as a linear function of the applied N ($y = 0.99x + 4.37$; $R^2 = 0.857$***) and, regardless of the amount of urea applied, total urea-N losses averaged only about 0.8 to 1.1% (Table 3.2).

Table 3.2. Cumulative NH_3 emissions from field soils amended with surface-applied urea at three rates (i.e., 0, 46, 92 kg N ha^{-1}). Emission rates were measured during a 2-h deployment period from replicate (n = 4) chambers.

Treatment	Urea-N applied	NH_3 loss†	CV	Urea-N loss
	kg N ha^{-1}	mg N m^{-2}	%	%
Control	0	11.5 $\pm$ 2.4 c	20.5	- - -
Low N	46	35.9 $\pm$ 5.0 b	13.9	0.78 $\pm$ 0.11
High N	92	103.4 $\pm$ 19.9 a	19.2	1.12 $\pm$ 0.22

† Within columns, means followed by the same letter are not significantly different at the P = 0.05 level of probability.

3.6 Conclusions

A closed, dynamic flux chamber (CDFC) system was developed and tested for in-situ measurements of ammonia emissions in the field. In the bench-scale tests, recovery efficiencies increased with increasing exposure time, though with the longest exposure time (360 min) some of the gain in efficiency could be attributed to re-absorption of NH_3 by the "source" solution as a result of acidification. In the field test, it was observed that treatment differences could be detected using sampling times as short as 30 min. However, the data suggest that longer sampling times are required to establish constant atmospheric transfer conditions within the chamber. Consequently,

it is recommended that the chambers be deployed for 90 to 120 min when used in the field. All chamber-based systems used to measure NH_3 emissions also influence the factors that affect NH_3 emission rates (e.g., wind speed, temperature, and partial pressure of NH_3 within the chamber). Consequently, it is often difficult to extrapolate total NH_3 losses from measurements obtained using chamber-based systems (Sommer et al., 2004; Harper, 2005). For example, changing the system parameters by increasing the air speed within the chamber is likely to increase emission rates, which in turn would strongly affect the calculated total NH_3 loss. Nevertheless, chamber-based measurement systems such as the CDFC system described here are extremely useful for making relative comparisons; i.e., for detecting (i) differences in NH_3 emission patterns under field conditions and (ii) treatment-induced differences in NH_3 emissions in the field. The low cost and ease of use of the CDFC system makes it especially useful for small plot and multi-treatment studies as well as for studies conducted at remote sites. Future research should focus on calibrating the CDFC system against more established systems such as those employing micrometeorological methods (Pacholski et al., 2006) or wind tunnels (Miola et al., 2015).

3.7 Acknowledgements

We like to thank Frank Krijnen for his support and expertise in constructing the chambers. Additional thanks goes to Indrajith Wickramananda for his support with the NH_3 analysis, and to Jay Bauer, David Bulmer, and Lori Pauls for their support during field sampling. We would also like to thank the Saskatchewan Forage Seed Development Commission, Agriculture and Agri-Food Canada, and the Agricultural Development Fund for their financial support.

4 FERTILIZER-INDUCED EMISSIONS OF AMMONIA AND NITROUS OXIDE FROM FORAGE SEED PRODUCTION IN THE BOREAL TRANSITION ZONE OF SASKATCHEWAN

4.1 Preface

While many studies have focused on the use of stabilized fertilizers in reducing gaseous N losses from annual crop production and grazed pasture systems, less is known about their use in forage seed production, especially in the Boreal Transition Zone of Saskatchewan. Consequently, this study was conducted to determine how stabilized fertilizers can reduce gaseous ammonia (NH_3) and nitrous oxide (N_2O) losses from perennial forage seed production. Gaseous N emissions from different stabilized fertilizers and urea were monitored in the field, utilizing the closed dynamic flux chamber system, previously described in Chapter 3.

4.2 Abstract

Forage seed production differs from forage feed production in that fertilizer management focuses on enhancing seed yield rather than plant biomass. In order to produce seeds, some forage grasses (e.g., hybrid bromegrass) require vernalization of the reproductive tillers, whereas others (e.g., timothy) do not. As a result, fertilizer N needs to be applied in either the fall or the spring, depending on the requirements of the crop. Furthermore, because forage grasses are perennial, fertilizer N cannot be incorporated easily and thus needs to be broadcast into the standing crop.

Urea is the most commonly used fertilizer for forage seed production in Saskatchewan, and its application to the soil surface can result in significant losses via ammonia (NH_3) volatilization and nitrous oxide (N_2O) emissions. For example, fall-applied urea fertilizers might be prone to N_2O losses, as the majority N_2O emissions in the Northern Great Plains occur in the spring during

snowmelt and thawing events. The higher temperatures after spring application, on the other hand, might result in significant losses via NH_3 volatilization.

Recently, stabilized fertilizers have shown promising results in mitigating gasous N losses. Stabilized N fertilizers contain either urease or nitrification inhibitors, or both, and their use is aimed at blocking key processes in the N-cycle that contribute to N losses (i.e., urea hydrolysis and nitrification). However, little is known about the efficacy of stabilized fertilizers in reducing gaseous N losses from forage seed production in the Boreal Transition Zone of Saskatchewan.

The aim of this study was to determine the efficacy of stabilized fertilizers, applied either in the fall or the spring, at reducing gaseous N_2O and NH_3 losses relative to untreated urea. Research plots were established using a randomized complete block design with existing forage seed production sites (n = 4), fertilizer products (n = 5) and time of application (n = 3) as the main factors. Fertilizers were applied in the fall of 2012, in the spring of 2013, and in the fall of 2013, and gaseous NH_3 and N_2O losses monitored after fertilizer application using chamber-based measurement systems. Treatments were an unfertilized control (C), urea (U), urea + urease inhibitor (UI), urea + nitrification inhibitor (NI), and urea + double inhibitor (DI). All four types were applied to deliver N at a rate of 92 kg N ha^{-1}.

Stabilized fertilizers containing urease inhibitors (UI and DI) reduced NH_3 emissions during the spring of 2013 and were more efficient at higher pH and when the soil was more moist. Although emissions were lower in the fall of 2013, stabilized fertilizers containing urease inhibitors showed a similar reduction in NH_3 losses. On the other hand, the effects of stabilized fertilizers on reducing N_2O emissions were mixed and differed between sites and timing of fertilizer application. For example, NI and DI reduced N_2O emissions from fall-applied fertilizers on all sites during snowmelt. After spring application, on the other hand, DI increased N_2O emissions at two sites, while emissions were strongly reduced or did not differ from untreated urea at the other two sites. However, DI demonstrated the largest potential in reducing gaseous N losses of both NH_3 and N_2O. It was concluded that the use of stabilized fertilizers, especially DI, would be most beneficial after spring application, when the potential for both NH_3 and N_2O losses is increased.

4.3 Introduction

Forage seed production requires significant fertility inputs that differ from forage feed production in that fertilizer strategies focus on seed rather than biomass production. Perennial forage grasses such as timothy (*Phleum pratense* L.) or bromegrass (*Bromus spp.* L.) have different induction requirements for flowering to produce seeds. Many forage grasses, including bromegrass, have a dual induction requirement for flowering (Heide, 1994). They need vernalization and/or short day length (SD) to initiate inflorescence primordia (primary induction), as well as long day

length induction (LD) to initiate culm elongation (secondary induction). Other forage grasses, including timothy, require only a single induction, usually LD, in order to initiate flowering (Heide, 1994). This physiological distinction between dual and single induced forage grasses matters when application of nitrogen (N) fertilizer for seed production is considered. For dual induced forage grasses, fertilizer strategies should include N application in the fall prior to cold temperature vernalization and before the onset of primary induction, to support formation of tillers suitable for seed production in the next year. In contrast, fertilizer strategies for single induced forage grasses should aim to apply fertilizer N before LD induction, usually in early spring. Consequently, the timing of fertilizer N application (i.e., fall or spring) is dependent on species-specific requirements for seed production. Bromegrass is a dual induced forage grass and thus can benefit from fall N application. Timothy is a single induced forage grass (Heide, 1994) for which the timing of fertilizer application is more flexible. Specifically, assuming N fertilizer losses are not significantly different between the two application times, both fall and spring application can be effective.

Forage grasses are perennial, typically grown for three or more years. Nitrogen fertilizers, therefore, cannot be incorporated into the soil after seeding and must be broadcast. Urea is the most commonly used fertilizer in the world, but its surface application can result in a variety of losses (Sommer et al., 2004; Glibert et al., 2006; IFA, 2014). Following enzymatic degradation of urea, gaseous ammonia (NH_3) can be lost through volatilization. Losses can reach levels greater than 50% of applied fertilizer N, depending on fertilizer type, climatic conditions, and soil properties (Sommer et al., 2004). Additionally, through microbially mediated nitrification, NH_3 can be rapidly converted to nitrate (NO_3^-) that is subject to leaching losses, or to gaseous nitrous oxide (N_2O) losses due to denitrification.

Nitrous oxide is a greenhouse gas with a global warming potential that is 298 times greater than that of CO_2 (Myhre et al., 2014). The amount and variation over time of NH_3 and N_2O emissions are dependent on the combination of many different physical, chemical, and biological factors and processes in the soil and at the soil surface. These include pH, moisture content, water-filled pore space, concentration of total ammoniacal N (TAN) in the soil solution, microbial activity, and wind speed. Furthermore, the presence of plant residues might increase urease activities and hydrolysis of urea, resulting in NH_3 losses (Rochette et al., 2009a). The contribution of each of these factors and processes to subsequent NH_3 and N_2O emissions varies from soil to soil, depending on their combination (Sherlock and Goh, 1984; Sommer et al., 2004; Cameron et al., 2013; Saggar et al., 2013b). Malhi et al. (2001) suggested that these N loss pathways generally reduce the nitrogen use efficiency (NUE) to less than 70% in temperate regions. Producers therefore need strategies that reduce N losses from broadcast applications of urea-based N.

One such strategy is to use urease and nitrification inhibitors together with fertilizer N application to block the transformation of urea to ammoniacal N (NH_3/NH_4^+), and ammoniacal N to

NO_3^-, respectively. Hydrolysis of surface-applied urea granules raises the pH, shifting the equilibrium of NH_3 and NH_4^+ in the soil solution towards NH_3 and thus favoring volatilization losses of NH_3 (Sommer et al., 2004). Urease inhibitors, such as N-(n-butyl) thiophosphoric triamide, can reduce these losses by inhibiting the transformation of urea to ammoniacal N for an extended period (Trenkel, 2010). This increases the time window during which precipitation can occur to help infiltrate the urea into the soil profile, thereby rendering it less susceptible to NH_3 volatilization losses. Nitrification inhibitors such as DCD inhibit the conversion of ammoniacal N to NO_3^-. Reducing the amount of N available as NO_3^- reduces the potential for NO_3^- leaching and N_2O emission losses.

Recent studies support the use of urease and nitrification inhibitors to reduce NO_3^- leaching, denitrification, and NH_3 volatilization losses (Di and Cameron, 2002; Zaman et al., 2008, 2009; Rochette et al., 2009a; Dawar et al., 2010; Engel et al., 2011). However, the majority of these studies were conducted under different climatic conditions than those in Saskatchewan. This study therefore evaluated the efficacy of stabilized urea fertilizers on mitigating gaseous NH_3 and N_2O losses when surface-applied to existing forage seed crops in northern Saskatchewan.

4.4 Materials and Methods

4.4.1 Test site selection and experimental setup

Four experimental sites were established within existing commercial forage seed fields in the Boreal Transition Zone of Saskatchewan near Carrot River (sites CR1 and CR2), Arborfield (site ABR), and Choiceland (site CHL). The fields were planted two to three years prior with hybrid bromegrass (*Bromus riparius* Rehm. $\times$ *Bromus inermis* Leyss.) at CR1 and CR2, and with timothy (*Phleum pratense*) at ABR and CHL. The sites CR1 ($53^\circ12'41''$N, $103^\circ30'21''$W) and CR2 ($53^\circ12'26''$N, $103^\circ30'41''$W) were located within the same field approximately 8 km south east of the town of Carrot River. Both sites were classified as Gleyed Dark Gray Chernozems of the Gronlid-Carrot River association, formed on a mixture of loamy lacustrine and sandy fluvial materials (Saskatchewan Land Resource Centre, 1997b), and differed in pH and soil organic matter (Table 4.1). The site ABR ($53^\circ10'52''$N, $103^\circ27'53''$W) was located 15 km southeast of the town of Carrot River and was classified as a Dark Grey Chernozem of the Melfort-Tisdale association, formed on clayey lacustrine materials (Saskatchewan Land Resource Centre, 1997a). The site CHL ($53^\circ28'49''$N, $104^\circ29'22''$W) was located 1 km south of Choiceland and was classified as a Gleyed Dark Gray Chernozem of the Kelsey-Garrick association, formed on a mixture of loamy glacial till and and silty lacustrine materials (Saskatchewan Land Resource Centre, 1997c).

Table 4.1. Location, forage crop, year of establishment, and soil properties of the four test sites.

Site	Location	pH	OC†	SOM‡	Crop	Established
			— g kg^{-1} —			
CR1	53°12′41″N, 103°30′21″W	7.61	23.0	39.2	Hybrid bromegrass	2010
CR2	53°12′26″N, 103°30′41″W	6.87	38.0	64.7	Hybrid bromegrass	2010
ABR	53°10′52″N, 103°27′53″W	5.43	35.0	61.0	Timothy	2009
CHL	53°28′49″N, 104°29′22″W	6.50	36.0	61.5	Timothy	2011

† OC = Organic carbon.

‡ SOM = Soil organic matter.

At each site, an experiment using a randomized complete block design with nine treatments and four replicates was established. Treatment plots measured 10.5 m wide × 11.2 m long. One treatment was the unfertilized control (C), the remaining eight treatments were surface applications of four different stabilized urea fertilizer types, applied in either the fall or spring. The four fertilizer types were: urea alone (U); urea coated with Agrotain® at the recommended rate of 1.5 g kg^{-1} urea, a formulation containing the urease inhibitor NBTPT (UI); SuperU™, a urea fertilizer containing both the urease inhibitor NBTPT and the nitrification inhibitor DCD (DI); and Alzon®, a urea fertilizer containing the nitrification inhibitor DCD (NI). All four types were applied at a rate of 92 kg N ha^{-1} (Table 4.2).

Table 4.2. Properties of different stabilized fertilizer products and urea used as the treatments in the field study.

Product. ID	Product	Inhibitor type	Active ingredient†	Mode of application‡
C	- - -	- - -	- - -	- - -
U	Urea	- - -	- - -	- - -
UI	Agrotain®	Urease inhibitor	NBTPT	Surface coated§
NI	Alzon®	Nitrification inhibitor	DCD + TZ	Incorporated
DI	SuperU™	Dual inhibitor	NBTPT + DCD	Incorporated

† NBTPT = N-(n-butyl) thiophosphoric triamide; DCD = dicyandiamide; TZ = 1H-1,2,4-triazole.

‡ The inhibitors were either incorporated into the fertilizer granules by the manufacturer during fertilizer production or coated onto the surface of the urea granules.

§ The product Agrotain® was coated onto the urea granules at the recommended rate of 1.5 g kg^{-1} urea.

Ammonia emissions were measured using a closed dynamic chamber system, adopted from Kissel et al. (1977) and Lockyer (1984), and N_2O emissions were measured using vented emission chambers according to Yates et al. (2006). Measurements of NH_3 emissions were conducted only at CR1 and CR2, due to the higher sampling resolution and labor intensity. Nitrous oxide emissions, however, were measured at all four sites. The chambers were located within the treatment plots on designated areas that had been covered during fertilizer application and did not initially receive fertilizer. The base frames of the emission chambers were inserted into the soil within the center of each treatment plot and remained for the whole measurement season. Fertilizer granules were applied by hand to the soil surface within the base frames in either the fall of 2012 or the spring of 2013 (Table 4.3). Ammonia and N_2O emissions from fall-applied fertilizers were monitored from immediately after fertilizer application to snowfall in 2012. Monitoring resumed as soon as the field was accessible following snowmelt in 2013 (i.e., May 6^{th}). Monitoring of spring-applied fertilizer emissions began immediately after application and ended when gas fluxes became negligible. Because of the extremely wet conditions that occurred in the fall of 2012, and which hampered sampling, fertilizer application and monitoring of gaseous N emissions at sites CR1 and CR2 (Table 4.3) were repeated in the fall of 2013.

Table 4.3. Time of fertilizer application and gas sampling period.

Site	Fertilizer applied	NH_3 emission sampling period	N_2O emission sampling period
		2012	
CR1	10 Oct. 2012	9 Oct. to 18 Oct.	4 Oct. to 19 Oct.
CR2	10 Oct. 2012	9 Oct. to 18 Oct.	4 Oct. to 19 Oct.
ABR	10 Oct. 2012	—	4 Oct. to 19 Oct.
CHL	10 Oct. 2012	—	4 Oct. to 19 Oct.
		2013	
CR1	10 Oct. 2012	7 May to 14 May	6 May to 27 May
CR2	10 Oct. 2012	7 May to 14 May	6 May to 31 May
ABR	10 Oct. 2012	—	6 May to 27 May
CHL	10 Oct. 2012	—	7 May to 27 May
CR1	23 May 2013	23 May to 3 June	23 May to 12 June
CR2	23 May 2013	23 May to 3 June	23 May to 12 June
ABR	15 May 2013	—	16 May to 20 June
CHL	15 May 2013	—	16 May to 20 June
CR1	18 Sept. 2013	18 Sept. to 25 Sept.	18 Sept. to 8 Oct.
CR2	18 Sept. 2013	18 Sept. to 25 Sept.	18 Sept. to 8 Oct.
ABR	—	—	—
CHL	—	—	—

4.4.2 Soil NH_3 emission measurements

Soil NH_3 emissions were measured using a closed dynamic chamber system consisting of a rectangular acrylic (6.35 mm thickness) base frame (46 cm $\times$ 22 cm $\times$ 15.25 cm; width $\times$ length $\times$ height) and a lid containing a battery-powered air pump and a fan. The chambers subsequently were installed into the soil to a depth of 5.25 cm, resulting in a headspace of 10.12 L. When the lid was closed, the inlet and outlet of the lid were connected to a vial holding 35 mL of a 0.245 *M* H_2SO_4 solution. An air pump and fan guided the air in the headspace of the chamber through the sulfuric acid solution at a speed of 2 L min^{-1}. Ammonia was retained in the acid solution while NH_3-free air was returned to the chamber. The lid, with air pump and fan, was closed only during

sampling. Initially, sampling duration was limited to 30 min d^{-1} during the fall of 2012. For all subsequent measurements, the system was improved by changing to a more energy-efficient pump and adding another battery slot. Emissions in all subsequent measurement seasons (i.e., spring and fall of 2013) were then sampled for 90 minutes per day. Daily sampling started at 1200 h on one site and at 1400 h on the other. The starting site was switched each day to limit any bias due to sampling time on emission patterns. After sampling, the sulfuric acid solutions were transferred into 50 mL Falcon tubes and transported back to the lab for analysis. Concentration of NH_3 within the acid solutions was determined colorimetrically using a SmartChem 200 autoanalyzer (Westco Scientific Instruments Inc., CT, USA). Fluxes were taken as the mass of NH_3-N captured in the acid trap divided by the duration of sampling. Cumulative emissions were calculated as the area under the curve between fluxes of all sampling points, based on the assumption that emission rates remained constant throughout the sampling day.

4.4.3 Soil N_2O emission measurements

Soil N_2O emissions were measured using vented emission chambers, according to Yates et al. (2006). The system consisted of a circular polyvinyl chloride base frame and a vented cap with a rubber sampling port. When the lid was closed, the chamber had a head space of 2.25 L and covered an area of 0.02 m^2. The base frames were inserted into the soil and remained there throughout the entire sampling period. The lid was only deployed during sampling events. Gas samples were drawn using a 25-mL syringe with a 25-gauge needle and were injected into 12-mL Exetainer™ tubes (Labco Limited, UK). Samples were drawn at 10 (t_{10}), 20 (t_{20}), and 30 (t_{30}) min after the lid was closed. An additional six to eight ambient air samples were drawn in pairs before the measurement on each site and the average was regarded as t_0. Samples were transported back to the lab and N_2O concentrations determined using a Bruker 450 GC gas chromatograph (Bruker Biosciences Corporation USA), according with Farrell and Elliott (2007).

Fluxes were calculated by fitting either an exponential or linear regression to the concentration *vs.* time data. Paired ambient air samples were used to calculate the minimal detectable concentration difference (MDCD), according to Yates et al. (2006). When concentrations of subsequent samples (i.e., at t_{10} and t_{20}) did not exceed the MDCD, they were regarded as not significantly different from each other and a linear regression was fitted to the data. When the concentration of subsequent samples exceeded the MDCD, a Hutchinson Mosier model (Hutchinson and Mosier, 1981; Pedersen et al., 2010; Pedersen, 2015) was fitted to the data. Fluxes were taken as the slope of either the linear regression or the Hutchinson Mosier model at t_0. Cumulative emissions were calculated by interpolating flux values of adjacent sampling points (Pennock et al., 2006).

4.4.4 Soil moisture content measurements

Volumetric soil moisture content (VMC%) was measured at sites CR1 and CR2 on each sampling day and experimental plot, using a portable TDR field probe. Soil moisture content was measured in the spring and fall of 2013. On each measurement plot, seven consecutive measurements were taken in distance of approximately 15 cm from the emission chambers, and the values averaged.

4.4.5 Measurement of snow water equivalent

A snow survey was conducted in February 2013 to assess the potential snow water equivalent (SWE) at each site. At each site, two diagonal transects of 10 sampling points each were established. At each sampling point, five depth measurements were conducted and averaged, and one snow core of 10 cm diameter was taken using a metal tube pushed into the snow surface and weighed. The snow water equivalent was calculated by multiplying the average snow depth with its density. Values were then averaged per site.

4.4.6 Calculations and statistical analyses

All calculations and statistical analyses were carried out using "R" (Version 3.0.2) (R Foundation for Statistical Computing, 2014). Cumulative fluxes were calculated by integrating the area under the curve of flux sampling points using the "flux 0.3-0" package in R (Jurasinski et al., 2015), which uses linear interpolation between non-sampling points (Pennock et al., 2006). Normality of the data and homogeneity of variance were tested using Shapiro Wilk's test and Levene's test, respectively. Data that were not normally distributed were transformed using a log transformation. A three-way ANOVA with fertilizer product, test site, and application timing (i.e., fall or spring) was conducted on cumulative fluxes of NH_3 or N_2O. The measurements of N_2O fluxes conducted in the fall of 2013 were excluded from this analysis, because snowmelt induced emissions could not be measured in the spring of 2014 due to logistical reasons. Post hoc Tukey's HSD test was used to determine the direction of significant differences.

4.5 Results

4.5.1 Weather and soil conditions

After fall N application in 2012 on day of the year (DOY) 284, average air temperatures for the measurement season (DOY 278 to 293) were generally low (3.2°C), with maximum temperatures of 18.2°C on DOY 289 (Fig. 4.1). Air temperatures after fall application in 2012 quickly fell below 0°C and remained low until the spring of 2013. Total precipitation during the fall 2012

measurement season was generally low, but showed one large precipitation event (25.6 mm) on DOY 291.

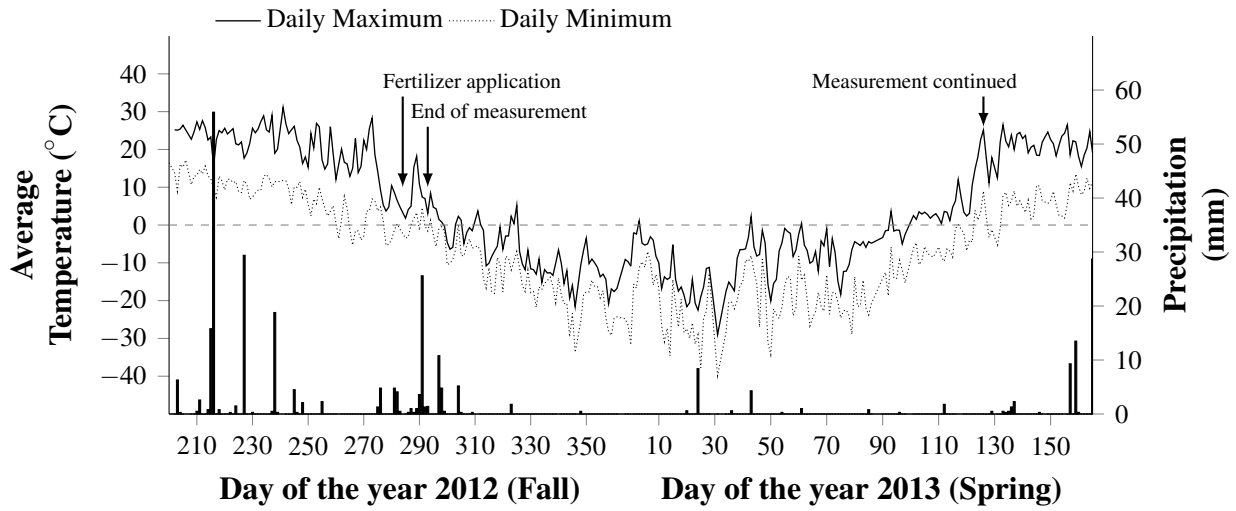


Fig. 4.1. Temperature and precipitation between fall 2012 and spring 2013 (Environment Canada, 2014).The measurement station was located near Nipawin, Canada.

Precipitation during the winter of 2012/2013 resulted in similar snowpack among all sites, ranging from 42.6 mm SWE at ABR, to 51.3 mm of SWE at CR2 (Table 4.4). Snowmelt in the spring of 2013 provided the soil with early-season moisture. As a result, the field sites were not accessible before DOY 126 due to the water-saturated soil conditions. Due to logistical reasons, volumetric water content of the soil could only be measured on sites CR1 and CR2. The site CR1 had a lower elevation than site CR2 and formed an area of standing water within the site during snowmelt. As a result, the average soil volumetric water content at CR1 was consistently higher than at CR2 throughout all measurement seasons (Table 4.4).

Table 4.4. Snow water equivalent (SWE) and soil volumetric water content (VWC) of the research sites.

Site	SWE (mm)	Soil VWC (%)		
		Post-snowmelt†	Spring 2013‡	Fall 2013§
CR1	46.2 ± 5.4	37.6 ± 1.9	29.2 ± 2.4	28.4 ± 5.8
CR2	51.3 ± 3.2	32.9 ± 3.8	26.1 ± 1.3	22.6 ± 5.6
ABR	42.6 ± 9.0	—	—	—
CHL	49.7 ± 6.1	—	—	—

† Measurement period from DOY 129 to 151.

‡ Measurement period from DOY 143 to 163.

§ Measurement period from DOY 261 to 281.

During the measurement period after N application in the spring of 2013 (DOY 143 to DOY 171), the average air temperature remained relatively constant at 14.5 ± 2.0°C (Fig. 4.2). Precipitation was limited, except for a few major events on DOY 157 (9.3 mm), DOY 159 (13.5 mm), and DOY 165 (28.7 mm).

In the fall of 2013, N application was conducted earlier (i.e., on DOY 261 to 281) than in the previous year (i.e., on DOY 278 to 293). As a result, average air temperatures during the fall 2013 measurement period were higher (i.e., 9.6°C) than in the fall of 2012 (i.e., 3.2°C).

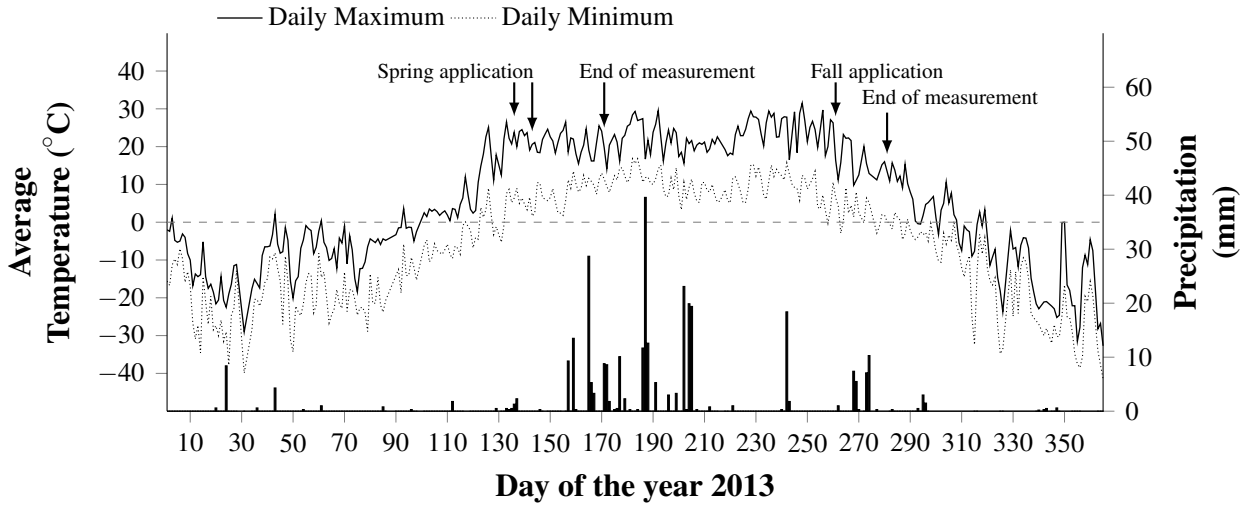


Fig. 4.2. Temperature and precipitation in 2013 (Environment Canada, 2014).The measurement station was located near Nipawin, Canada.

4.5.2 Effect of timing of fertilizer application on gaseous N losses

Nitrous oxide emissions from surface applied fertilizers had a seasonal response, as the majority of N_2O emissions from fall-applied fertilizers occurred in the spring of 2013 after snowmelt at all four sites (Figs. 4.3 to 4.5). Fall emissions were relatively low (0 to 19.8 g N ha^{-1} d^{-1}) throughout the measurement period, and essentially ceased after snowfall and low temperatures reduced N_2O emissions. Immediately after snowmelt in the spring of 2013 (DOY 126), nitrous oxide emissions reached their highest levels (49.9 to 317.1 g N ha^{-1} d^{-1}) and decreased within the next four days on all sites, except CHL, for which the initial emission peak was absent. This decrease in emissions was accompanied by a drop in mean air temperature (17.1 to 3.9°C) after DOY 126. After DOY 135, the air temperature stayed relatively constant at 11 to 15°C, as emissions declined until the end of the spring measurement period.

Ammonia emissions from fall-applied urea showed a trend opposite to N_2O emissions, as the majority of NH_3 emissions occurred in the fall of 2012 directly after fertilizer application (Figs. 4.3 and 4.4). Ammonia emissions peaked 5 d after application (DOY 289) and were generally low (25.3 to 56.4 g N ha^{-1} d^{-1}). Due to a precipitation event on DOY 291 and a drop in mean air temperature, emissions declined and measurement was suspended after DOY 293. In the spring of 2013, NH_3 emissions from fall-applied urea were negligible (i.e., not different from the unfertilized control), likely as a result of movement of urea into the soil.

Application of fertilizers in the spring of 2013 generally resulted in increased N_2O emissions (0 to 92.4 g N ha^{-1} d^{-1}) relative to fertilizers applied in the fall at all sites (Figs. 4.6 to 4.8). Emissions at CR2, however, were only as low as during the fall measurements. Emissions peaked within 4 to 6 d after fertilizer application and decreased within the next 7 d, except at CHL, where emissions reached a second peak at DOY 147 to 149.

Ammonia emissions from spring-applied fertilizers (Fig. 4.6 and 4.7) were up to five times higher (1.5 to 517.1 g N ha^{-1} d^{-1}) than from fertilizers applied in the fall of 2012 and differed among sites. Maximum emissions at the high-pH site, CR1 (517.1 g N ha^{-1} d^{-1}), were three times higher than at the low-pH site, CR2 (174.4 g N ha^{-1} d^{-1}). At both sites, the emissions peaked 6 d after fertilizer application (DOY 149). One day after the highest emission rates were observed, emissions decreased, remaining low until the end of the measurement season, although slightly above the unfertilized control (1.2 to 14.7 g N ha^{-1} d^{-1}).

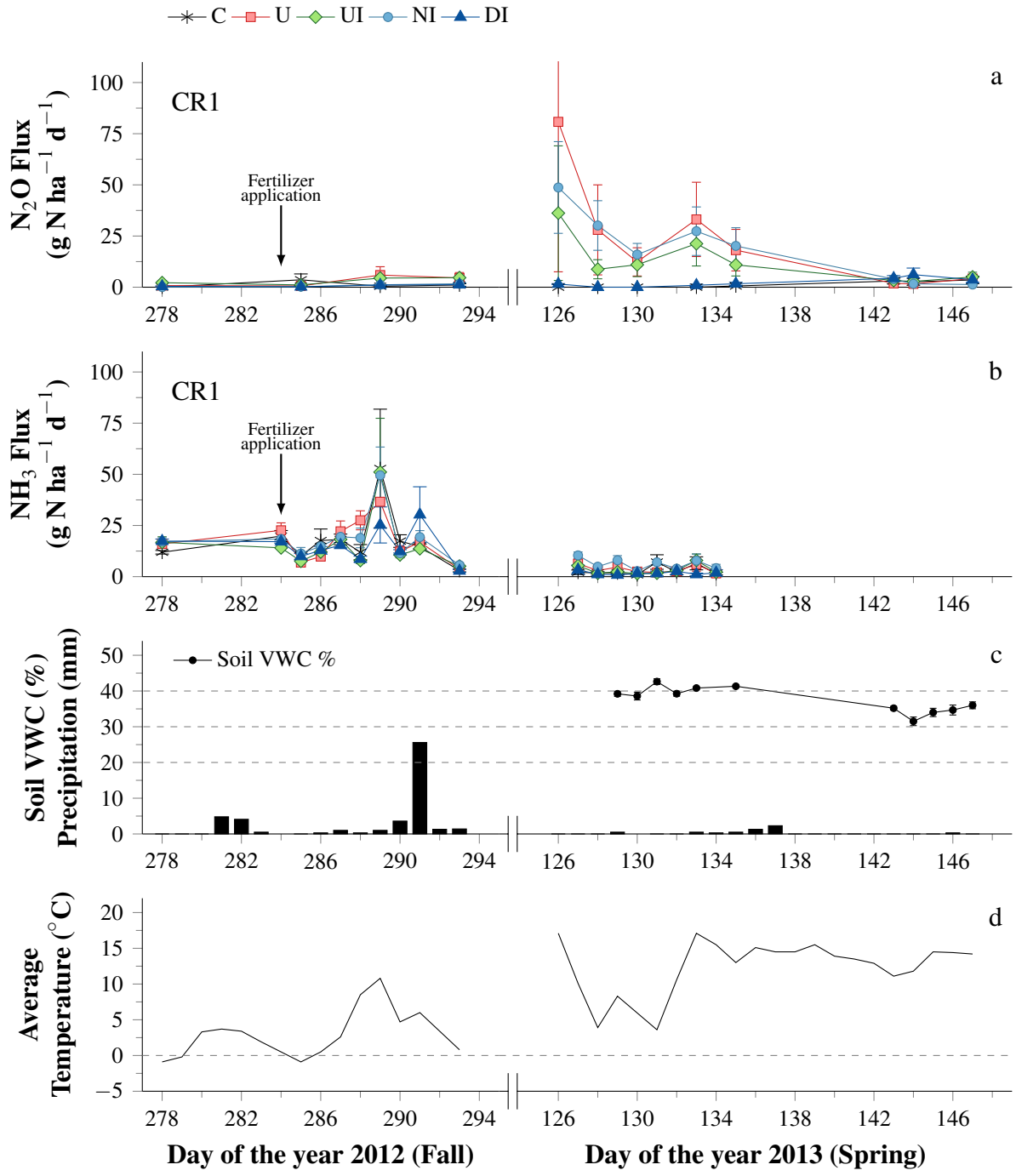


Fig. 4.3. Soil emissions of N_2O (a) and NH_3 (b) in relation to precipitation and soil volumetric water content (c) and air temperature (d) from fertilizers applied in the fall of 2012 at site CR1. Error bars represent standard error of the mean. C = unfertilized control, U = untreated urea, UI = urease inhibitor (NBTPT), NI = nitrification inhibitor (DCD + TZ), DI = double inhibitor (NBTPT + DCD).

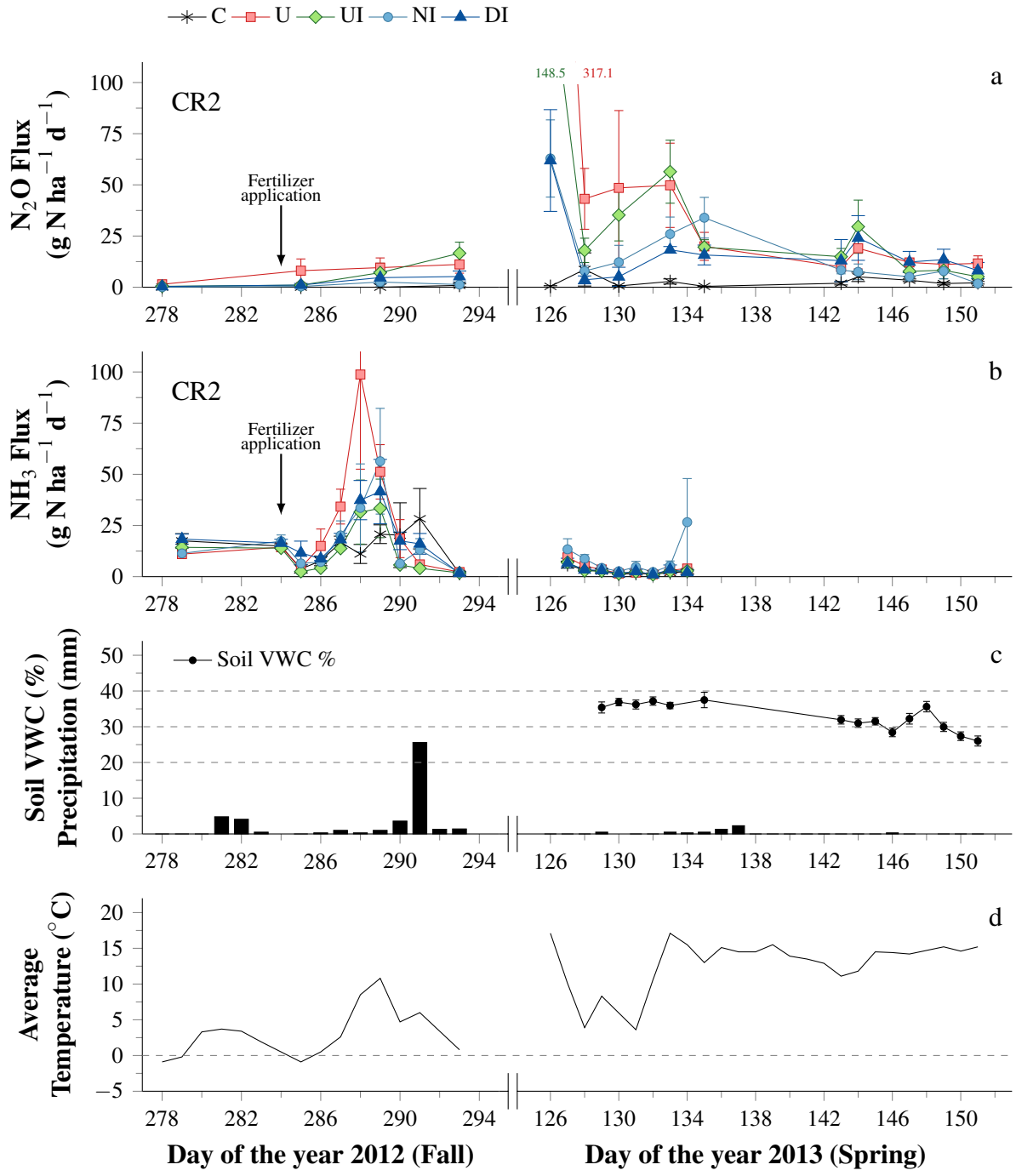


Fig. 4.4. Soil emissions of N_2O (a) and NH_3 (b) in relation to precipitation and soil volumetric water content (c) and air temperature (d) from fertilizers applied in the fall of 2012 at site CR2. Error bars represent standard error of the mean. C = unfertilized control, U = untreated urea, UI = urease inhibitor (NBTPT), NI = nitrification inhibitor (DCD + TZ), DI = double inhibitor (NBTPT + DCD).

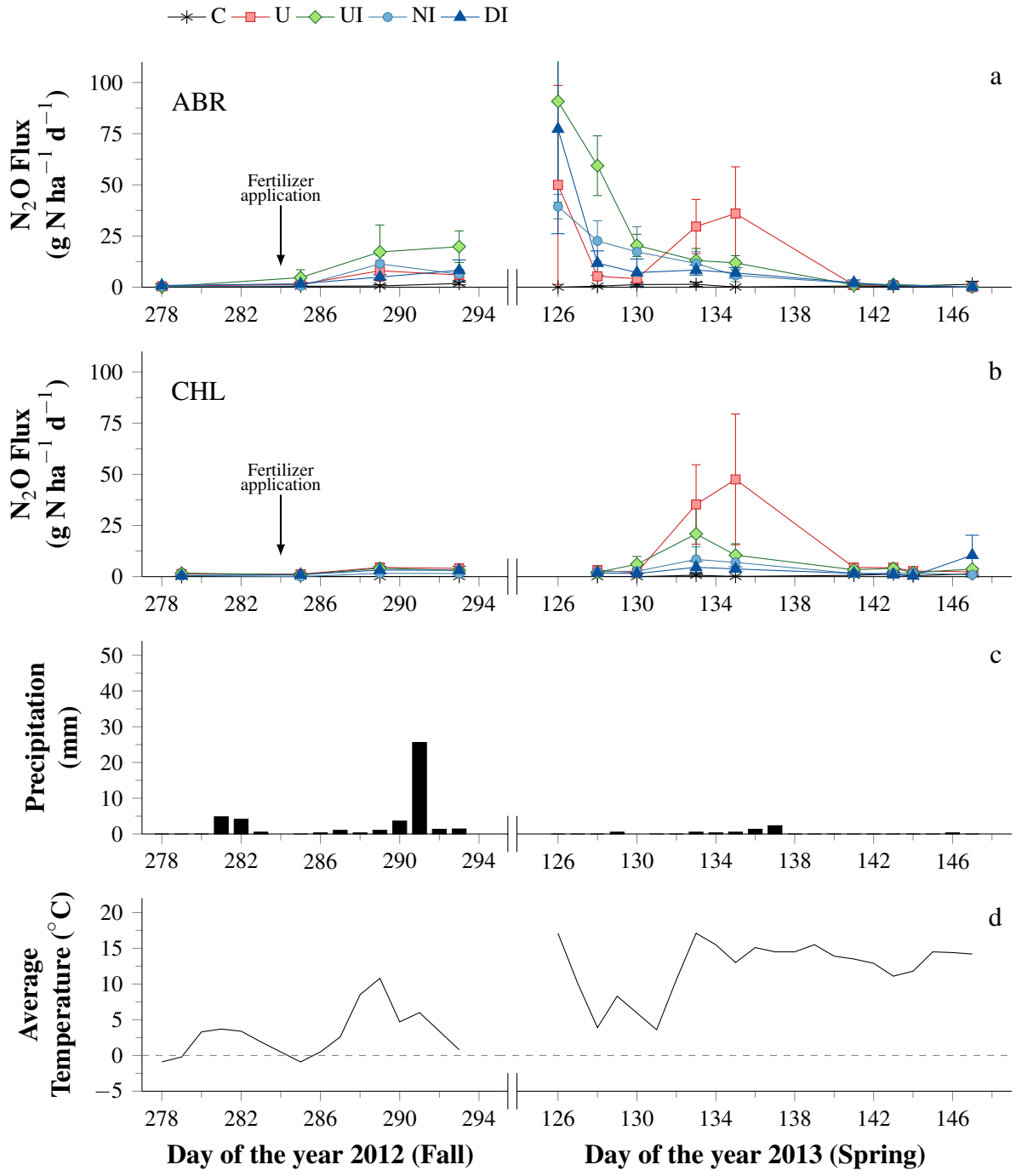


Fig. 4.5. Soil emissions of N_2O at ABR (a) and CHL (b) in relation to precipitation (c) and air temperature (d) from fertilizers applied in the fall of 2012. Error bars represent standard error of the mean. C = unfertilized control, U = untreated urea, UI = urease inhibitor (NBTPT), NI = nitrification inhibitor (DCD + TZ), DI = double inhibitor (NBTPT + DCD).

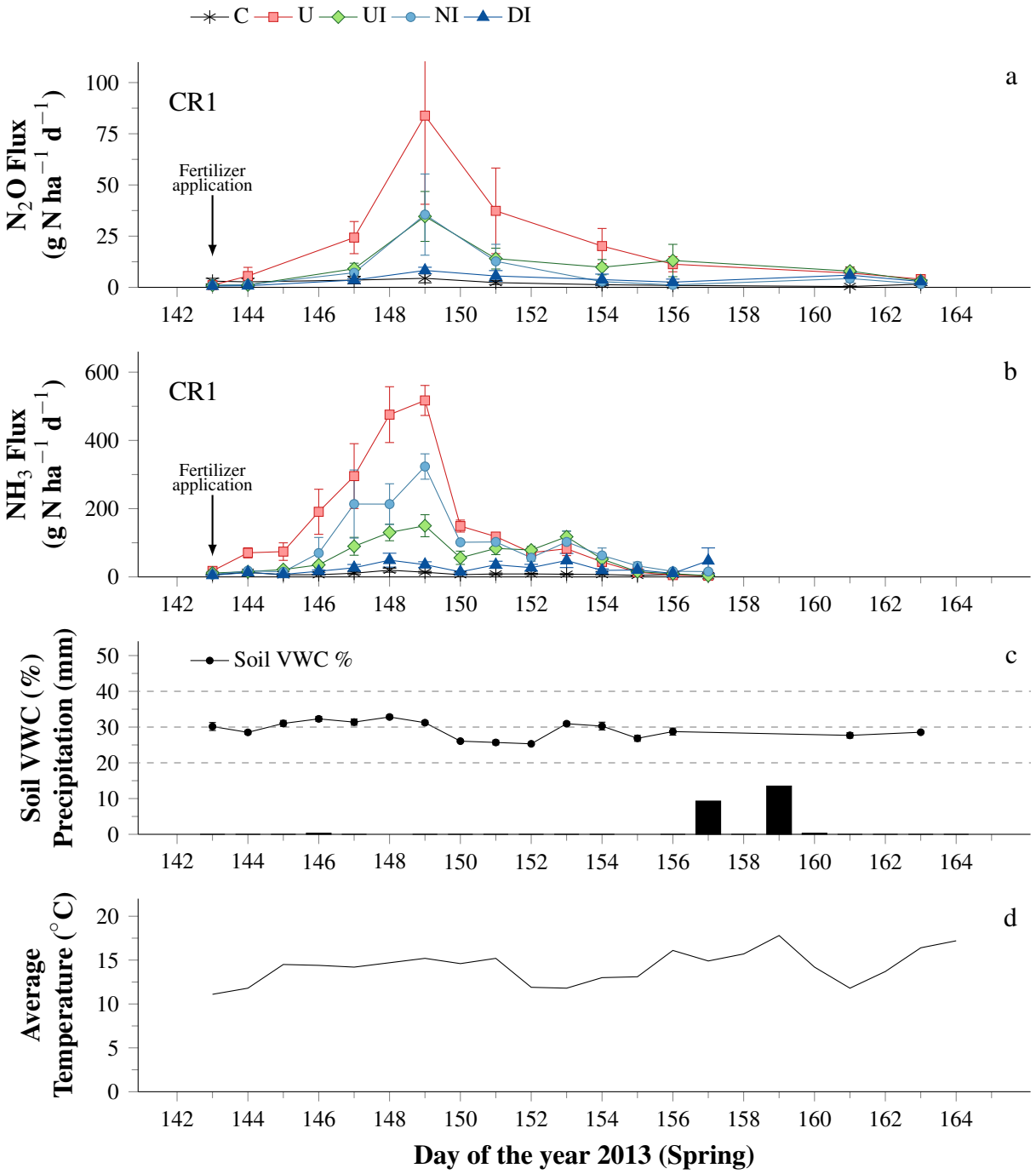


Fig. 4.6. Soil emissions of N_2O (a) and NH_3 (b) in relation to precipitation and soil volumetric water content (c) and air temperature (d) from fertilizers applied in the spring of 2013 at site CR1. Error bars represent standard error of the mean. C = unfertilized control, U = untreated urea, UI = urease inhibitor (NBTPT), NI = nitrification inhibitor (DCD + TZ), DI = double inhibitor (NBTPT + DCD).

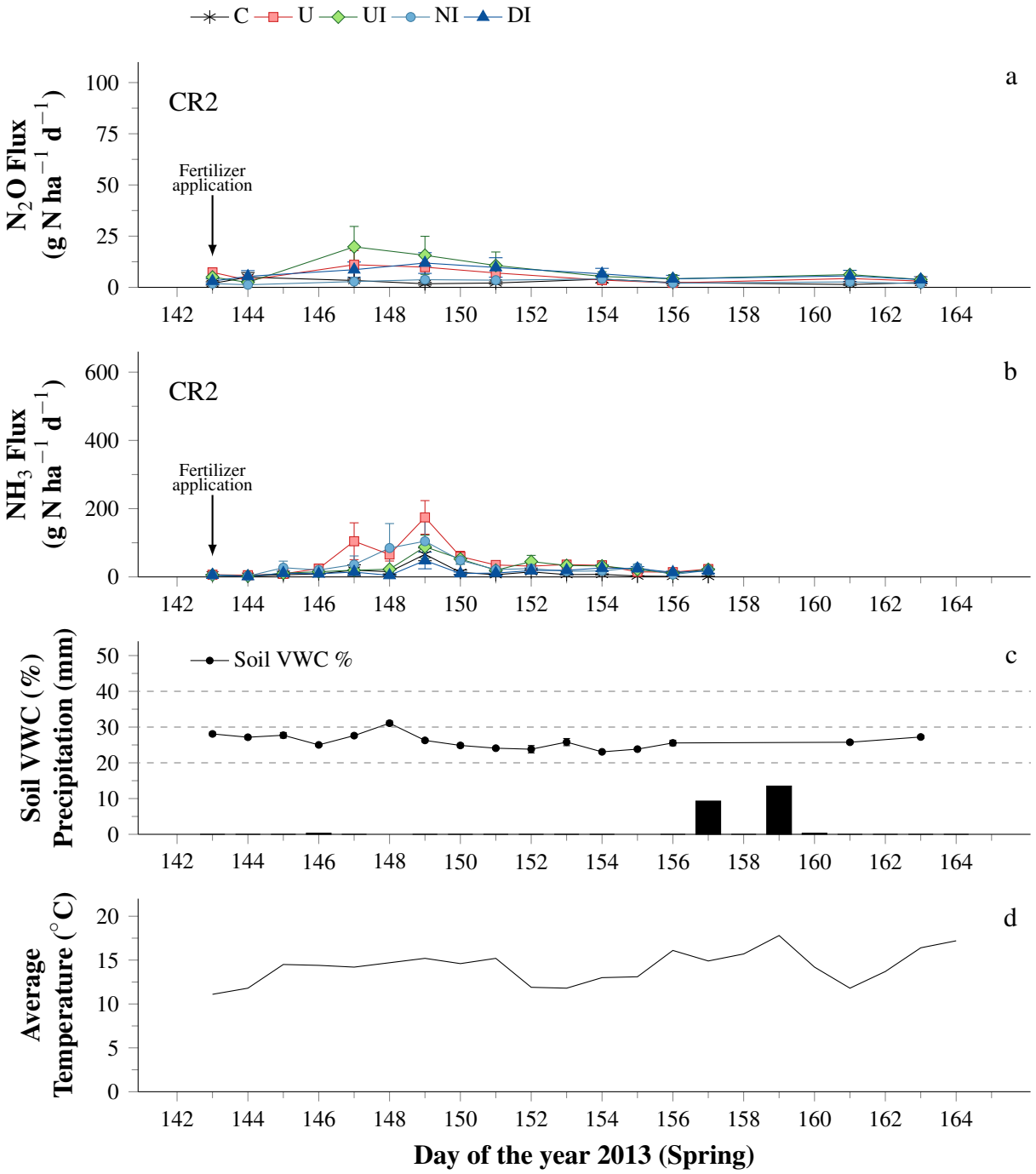


Fig. 4.7. Soil emissions of N_2O (a) and NH_3 (b) in relation to precipitation and soil volumetric water content (c) and air temperature (d) from fertilizers applied in the spring of 2013 at site CR2. Error bars represent standard error of the mean. C = unfertilized control, U = untreated urea, UI = urease inhibitor (NBTPT), NI = nitrification inhibitor (DCD + TZ), DI = double inhibitor (NBTPT + DCD).

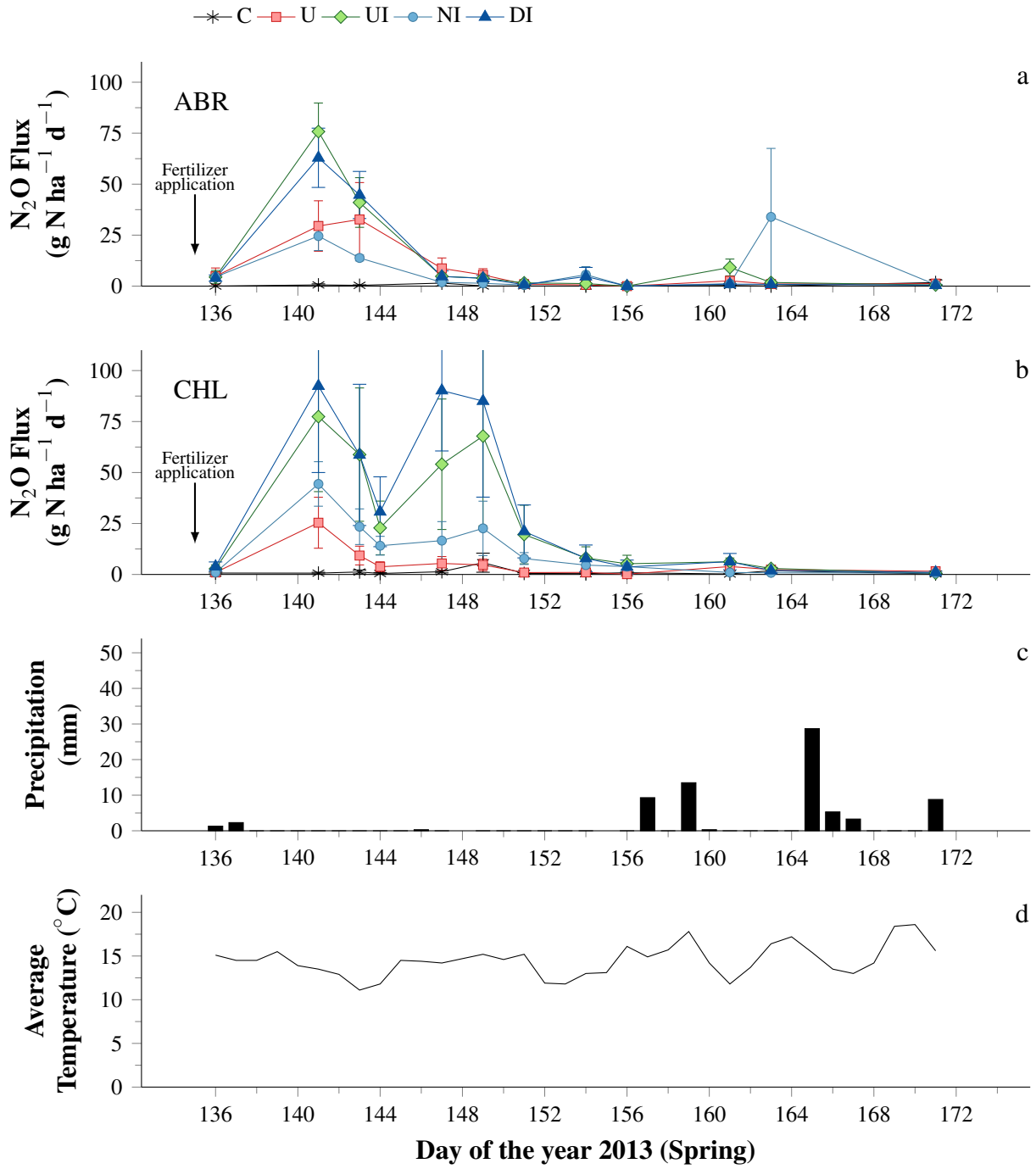


Fig. 4.8. Soil emissions of N_2O at ABR (a) and CHL (b) in relation to precipitation (c) and air temperature (d) from fertilizers applied in the spring of 2013. Error bars represent standard error of the mean. C = unfertilized control, U = untreated urea, UI = urease inhibitor (NBTPT), NI = nitrification inhibitor (DCD + TZ), DI = double inhibitor (NBTPT + DCD).

After fertilizer application in the fall of 2013 (Figs. 4.9 to 4.10), emissions of both N_2O and NH_3 generally showed a similar pattern to those observed in the previous fall (Figs. 4.3 to 4.4). Nitrous oxide emissions remained as low as in the previous fall (0 to 28.4 g N ha^{-1} d^{-1}). Emissions peaked on DOY 274, likely as a result of the precipitation events on DOY 273 and 274, and declined until the end of the measurement period (i.e., DOY 281).

Ammonia emissions from fertilizers applied in the fall of 2013 (Figs. 4.9 to 4.10) were also in the same order of magnitude as in the previous fall (2.1 to 100.4 g N ha^{-1} d^{-1}), although more high-emission days were observed in 2013, likely as a result of higher average air temperatures associated with an earlier measurement season. Emissions peaked 2 d after fertilizer application and rapidly decreased to levels of the unfertilized control after a precipitation event on DOY 268 at both CR1 and CR2, which caused a strong increase in soil moisture content (Figs. 4.9 to 4.10).

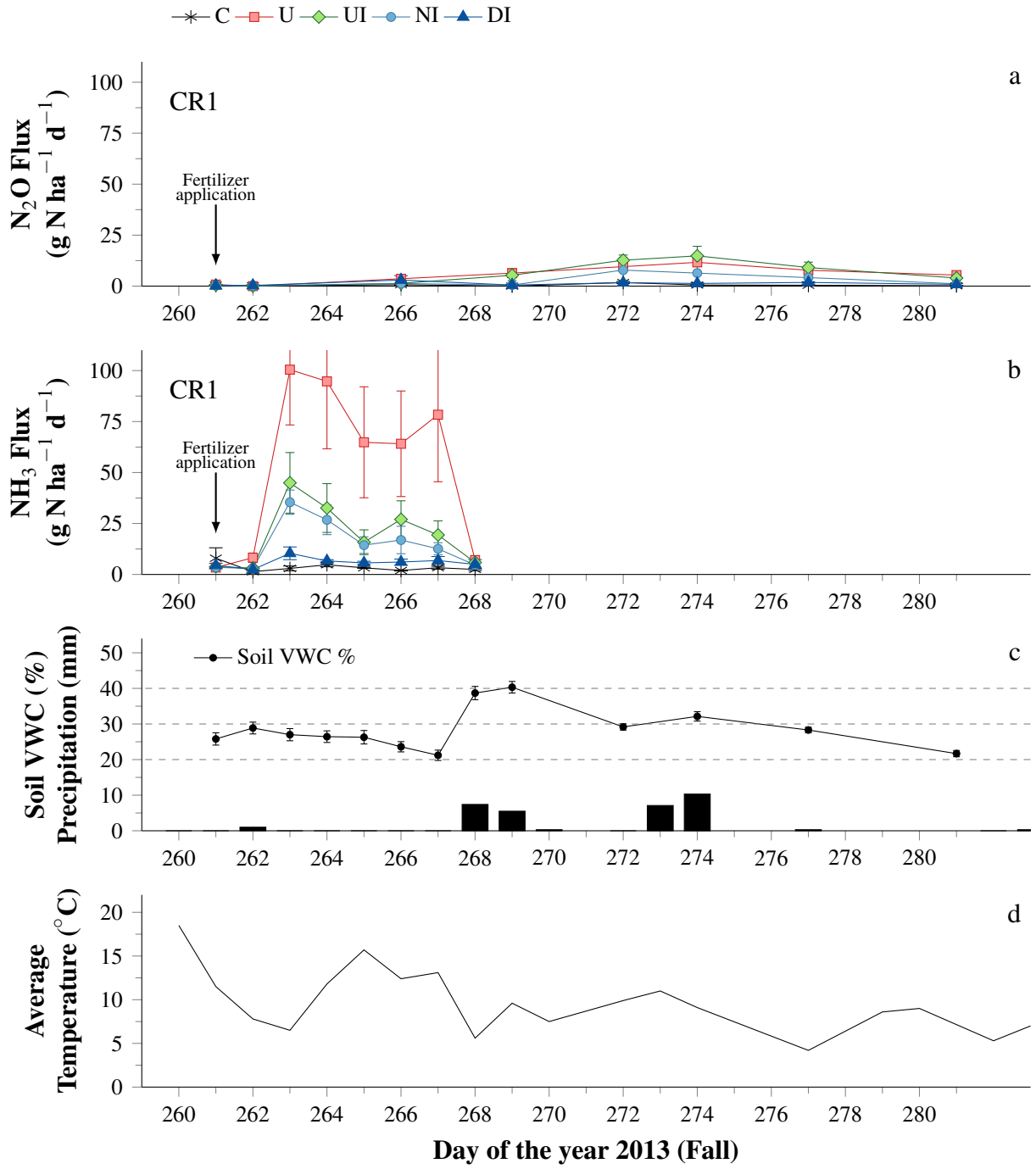


Fig. 4.9. Soil emissions of N_2O (a) and NH_3 (b) in relation to precipitation and soil volumetric water content (c) and air temperature (d) from fertilizers applied in the fall of 2013 at site CR1. Error bars represent standard error of the mean. C = unfertilized control, U = untreated urea, UI = urease inhibitor (NBTPT), NI = nitrification inhibitor (DCD + TZ), DI = double inhibitor (NBTPT + DCD).

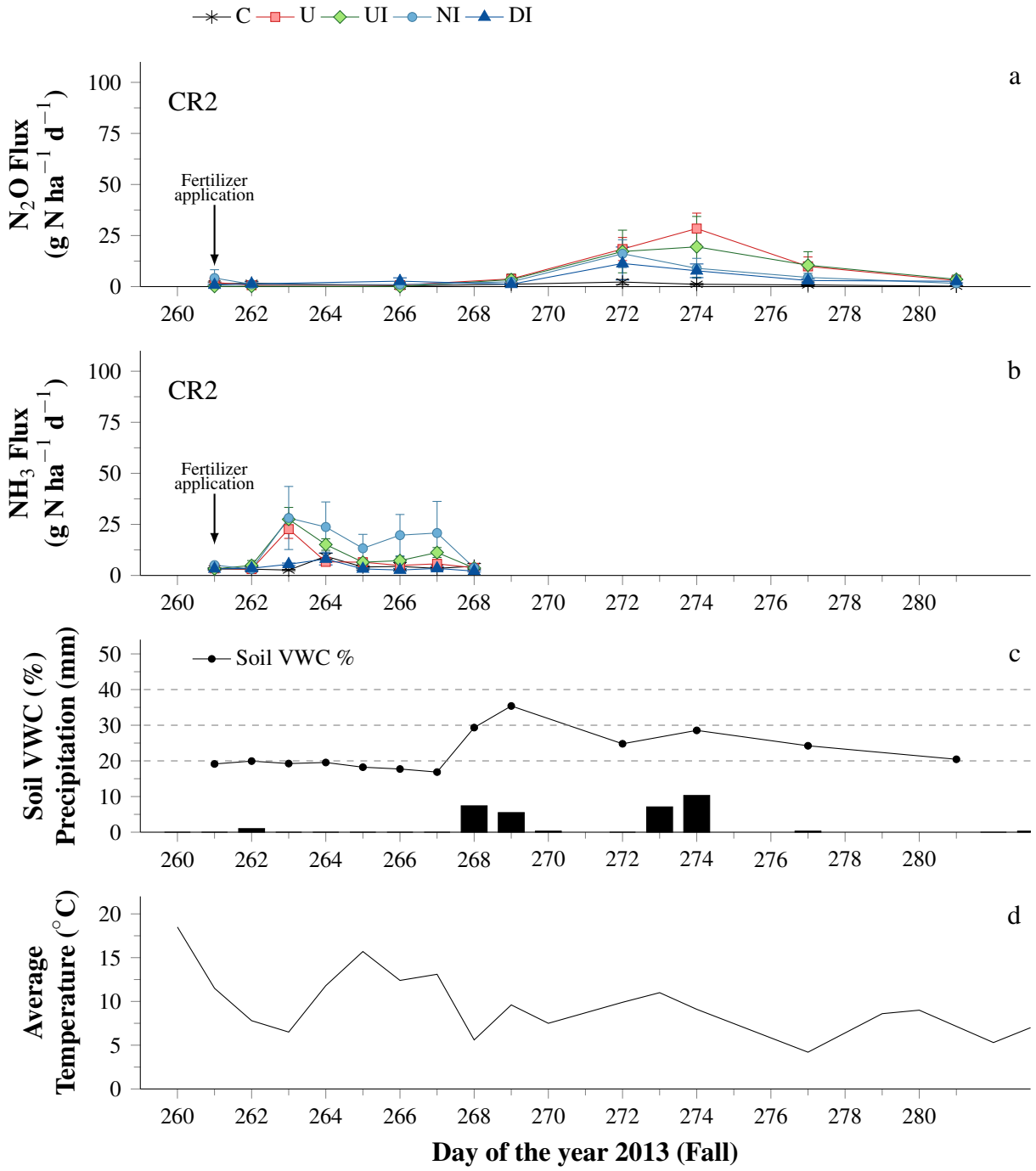


Fig. 4.10. Soil emissions of N_2O (a) and NH_3 (b) in relation to precipitation and soil volumetric water content (c) and air temperature (d) from fertilizers applied in the fall of 2013 at site CR2. Error bars represent standard error of the mean. C = unfertilized control, U = untreated urea, UI = urease inhibitor (NBTPT), NI = nitrification inhibitor (DCD + TZ), DI = double inhibitor (NBTPT + DCD).

4.5.3 Performance of stabilized fertilizers in reducing N_2O emissions

A factorial ANOVA revealed significant interactions between test site and application time, as well as between fertilizer treatment and test site (Table A.1); therefore, the data was separated by application time (i.e., fall 2012, spring 2013, and fall 2013) and a univariate ANOVA was conducted on cumulative N_2O losses for each site and application time.

In the fall of 2012, average daily N_2O emissions from fertilizers containing nitrification inhibitors (i.e., NI and DI) tended to be lower than those from urea (Table 4.5). Whereas this trend was apparent throughout the whole sampling period, but especially during the period immediately after snowmelt (DOY 126) when emissions from urea were greatest (Figs. 4.3 to 4.5), differences among the average daily fluxes were not significant (Table 4.5). At site CR2, for example, emissions from urea were as high as 317.1 g N ha^{-1} d^{-1}, whereas emissions from the NI and DI reached levels of only 61.7 g N ha^{-1} d^{-1} and 60.8 g N ha^{-1} d^{-1}, respectively. A similar effect was observed at site CR1, though peak emissions from the urea were considerably lower (80.8 g N ha^{-1} d^{-1}) than those observed at CR2. The product containing the urease inhibitor (UI), on the other hand, tended to produce N_2O emissions that were smaller than those from urea, but greater than those from products containing nitrification inhibitors. In general, average daily emission rates tended to increase in the order: DI and NI < UI < urea (see Table 4.5). However, the data also suggest that the N_2O emissions were associated with a large degree of spatial variability that tended to obscure differences between products (i.e., though fairly consistent, differences were generally not significant). As a result, average daily N_2O emissions from fall-applied fertilizers did not differ significantly. Despite the low over-all emissions after fertilizer application in the fall of 2013, emissions from the various fertilizer products followed the same order as in the previous fall (Table 4.5).

Table 4.5. Average daily N_2O emissions from fertilizers applied in the fall of 2012, in the spring of 2013, and in the fall of 2013.

Site	Treatment†	Fall-applied (2012)‡		Spring-applied (2013)§	Fall-applied (2013)¶
		g N ha^{-1} d^{-1}#			
CR1	C	1.3 ± 1.0	(46.8%)	1.9 ± 1.4 b	0.5 ± 0.2 b
	U	10.5 ± 10.4	(9.8%)	21.5 ± 18.4 a	5.9 ± 1.1 a
	UI	6.8 ± 5.7	(15.9%)	11.0 ± 7.0 a	6.2 ± 2.7 a
	NI	9.2 ± 6.3	(2.7%)	7.2 ± 5.8 ab	2.8 ± 3.4 ab
	DI	1.5 ± 0.5	(16.1%)	4.0 ± 1.7 ab	1.3 ± 0.9 b
CR2	C	1.5 ± 0.9 b	(2.9%)	2.5 ± 0.4	1.0 ± 0.6 b
	U	23.7 ± 14.2 a	(11.0%)	5.2 ± 4.0	8.3 ± 4.7 a
	UI	17.6 ± 8.5 a	(9.3%)	8.0 ± 6.7	7.0 ± 8.4 ab
	NI	10.3 ± 5.1 a	(3.4%)	2.6 ± 1.8	4.6 ± 3.8 ab
	DI	9.7 ± 4.1 a	(8.8%)	6.5 ± 5.2	3.8 ± 2.4 ab
ABR	C	0.6 ± 0.2 b	(37.8%)	0.4 ± 0.5	—
	U	9.1 ± 5.4 a	(14.5%)	7.6 ± 7.3	—
	UI	12.7 ± 7.2 a	(27.9%)	13.1 ± 5.6	—
	NI	6.7 ± 4.9 a	(24.7%)	9.4 ± 9.7	—
	DI	6.0 ± 2.0 a	(21.4%)	11.4 ± 5.2	—
CHL	C	0.3 ± 0.3 b	(9.7%)	1.1 ± 0.5 b	—
	U	9.4 ± 9.2 a	(10.2%)	4.8 ± 3.3 ab	—
	UI	4.6 ± 3.4 a	(18.6%)	22.5 ± 23.9 a	—
	NI	2.2 ± 1.7 a	(13.3%)	10.1 ± 6.0 ab	—
	DI	2.2 ± 1.7 a	(31.2%)	27.8 ± 23.1 a	—

† C = unfertilized control, U = untreated urea, UI = urease inhibitor (NBTPT), NI = nitrification inhibitor (DCD + TZ), DI = double inhibitor (NBTPT + DCD).

‡ Emissions were measured in the fall of 2012 (15 to 16 d) and in the spring of 2013 (21 to 24 d) immediately after snowmelt. Values in parentheses represent the percentage of emissions that occurred in the fall.

§ Emissions were measured for 21 d at CR1 and CR2, and for 36 d at ABR and CHL.

¶ Emissions were measured for 21 d at CR1 and CR2.

Within columns, means followed by the same letter are not significantly different at the $P = 0.05$ level of probability.

The efficacy of spring-applied fertilizers to reduce N_2O emissions did not differ statistically between fertilizer products; though products containing nitrification inhibitors (i.e., NI and DI) showed trends in average daily N_2O losses that differed between sites. For example, daily N_2O emissions from the urea were generally higher at CR1 ($\bar{x}$ = 21.5 g N ha^{-1} d^{-1}) than at CR2 ($\bar{x}$ = 5.2 g N ha^{-1} d^{-1}); however, whereas the NI and DI products were generally associated with lower emissions than the urea at CR1 ($\bar{x}$ = 7.2 g N ha^{-1} d^{-1} and 4.0 g N ha^{-1} d^{-1}, respectively) no such trend was observed at CR2. Interestingly, at the ABR and CHL sites, the products containing urease inhibitors (i.e., DI and UI) resulted in relatively larger N_2O losses compared to urea. Although the NI did not reduce N_2O emissions relative to urea at these sites, emissions from plots receiving the NI tended to be lower than those from plots receiving the UI or DI. This suggests that the presence of a urease inhibitor in these products could result in an increase in N_2O emissions. At the CHL site, for example, average daily emissions from the DI (27.8 g N ha^{-1} d^{-1}) and UI (22.5 g N ha^{-1} d^{-1}) generally exceeded those associated with the urea (4.8 g N ha^{-1} d^{-1}) and NI (10.1 g N ha^{-1} d^{-1}).

4.5.4 Performance of stabilized fertilizers in reducing NH_3 emissions

Data analysis revealed that the average daily NH_3 emissions were not normally distributed, even after log transformation, and that average daily emissions from each measurement season followed a different distribution. As a result, a two-way ANOVA was conducted for each fertilizer application time (i.e., fall 2012, spring 2013, fall 2013), with fertilizer treatment and test site as the main factors. Because there were signicant ($P = 0.008$) fertilizer $\times$ site interactions (see Tables A.2 and A.3) for both the spring 2013 and the fall 2013 fertilizer applications, emissions from these sites were analyzed using a univariate ANOVA.

Ammonia emissions from fertilizers applied in the fall of 2012 were not significantly different than those from the unfertilized control, and there were no significant differences between the stabilized products and urea (see Figs. 4.3 and 4.4). Fertilizers applied in the fall of 2013, on the other hand, resulted in ammonia emissions that exhibited strong fertilizer product and site effects. For example, NH_3 emissions at the CR1 site were greatest from the plots treated with urea and were significantly reduced in the plots treated with the stabilized NI and DI fertilizer products (Fig. 4.9b). Average daily emissions were lowest from DI (5.3 g N ha^{-1} d^{-1}) and did not differ signicantly from the unfertilized control (Table 4.6). Furthermore, average daily NH_3 emissions from UI (18.5 g N ha^{-1} d^{-1}) were not signicantly different from the untreated urea, but were signicantly higher than from DI. Interestingly, average daily emissions from NI (14.1 g N ha^{-1} d^{-1}) were as low as from UI, despite the absence of a urease inhibitor. At CR2, on the other hand, emissions from untreated urea were much lower than at CR1 (Fig. 4.10). As a result, stabilized fertilizers containing urease inhibitors (i.e., UI and DI) did not reduce average daily NH_3 emissions signicantly when compared to urea (Table 4.6).

Fertilizers applied in the spring of 2013 resulted in much greater NH_3 emissions than fall-applied fertilizers. Furthermore, emissions were much greater at CR1 than at CR2 (Figs. 4.6 and 4.7), which is similar to what was observed in the fall of 2013 (Figs. 4.9 and 4.10). At the CR1 site, all stabilized fertilizer products containing urease inhibitors (i.e., UI and DI) reduced NH_3 emissions compared to urea (Table 4.6). For example, average daily NH_3 emissions from the products containing urease inhibitors (i.e., UI and DI) were signicantly lower (57.5 and 23.3 g N ha^{-1} d^{-1}, respectively) than those from the untreated urea (141.2 g N ha^{-1} d^{-1}). Interestingly, emissions from the NI tended to be lower ($\bar{x}$ = 89.3 g N ha^{-1} d^{-1}) than those from the urea, paralleling a similar trend observed in the fall of 2013. At the CR2 site, where emissions from urea were much lower (41.5 g N ha^{-1} d^{-1}) than at CR1, emissions from DI and UI were numerically lowest (14.9 and 25.8 g N ha^{-1} d^{-1}), although these differences were not statistically signicant (P = 0.13 and P = 0.72, respectively).

Table 4.6. Average daily NH_3 emissions from fertilizers applied in the fall of 2012, in the spring of 2013, and in the fall of 2013.

Site	Treatment†	Fall-applied (2012)‡		Spring-applied (2013)§	Fall-applied (2013)¶
		g N ha^{-1} d^{-1}#			
CR1	C	15.6 ± 4.1	(92.1%)	8.3 ± 1.0 d	2.8 ± 1.1 c
	U	16.5 ± 4.7	(91.6%)	141.2 ± 22.5 a	52.0 ± 35.2 a
	UI	14.1 ± 4.4	(91.4%)	57.5 ± 7.2 b	18.5 ± 9.5 ab
	NI	17.6 ± 3.0	(86.9%)	89.3 ± 32.8 ab	14.1 ± 5.3 bc
	DI	14.2 ± 3.6	(95.5%)	23.3 ± 9.7 c	5.3 ± 1.5 c
CR2	C	12.7 ± 4.2	(93.0%)	11.6 ± 5.0	5.4 ± 0.5
	U	18.2 ± 6.2	(92.9%)	41.5 ± 20.3	6.6 ± 2.0
	UI	10.8 ± 0.8	(90.9%)	25.8 ± 13.8	9.5 ± 3.4
	NI	16.9 ± 2.4	(84.2%)	30.0 ± 25.4	14.1 ± 14.9
	DI	14.0 ± 1.8	(92.3%)	14.9 ± 4.5	3.6 ± 0.8

† C = unfertilized control, U = untreated urea, UI = urease inhibitor (NBTPT), NI = nitrification inhibitor (DCD + TZ), DI = double inhibitor (NBTPT + DCD).

‡ Emissions were measured in the fall of 2012 for 16 d and in the spring of 2013 immediately after snowmelt for 8 d. Values in parentheses represent the percentage of emissions that occurred in the fall.

§ Emissions were measured for 15 d at CR1 and CR2.

¶ Emissions were measured for 8 d at CR1 and CR2.

Within columns, means followed by the same letter are not significantly different at the $P = 0.05$ level of probability.

4.6 Discussion

4.6.1 Effect of weather conditions and timing of fertilizer application on gaseous N losses

Nitrous oxide emissions from fall-applied urea were dominated by large fluxes immediately after snowmelt in the spring of 2013, while emissions were generally low during the fall of 2012 and 2013. This is not surprising, as N_2O emissions are known to be sensitive to increases in water-filled pore space and increasing temperature during snowmelt events, which lead to ideal conditions for denitrification (Nyborg et al., 1997; Lemke et al., 1998; Dusenbury et al., 2008). Furthermore, because the sites were not accessible for sampling before DOY 126 in the spring of 2013, it is possible that the majority of snowmelt-induced N_2O emissions occurred in the period

between DOY 110 and 126, when temperatures rose above 0°C for the first time in the year and thus were not detected in this study (Fig. 4.1). This likely explains the absence of the initial post-snowmelt emission peak at CHL. Furthermore, site CR2, with a slightly higher SWE than CR1, showed higher post-snowmelt emissions compared to the latter site. This indicated that a relatively small difference in SWE might have a strong effect on post-snowmelt emissions of N_2O.

Nitrous oxide emissions in both the fall of 2012 and 2013, on the other hand, were low due to reduced denitrification activity as a result of low average air temperatures. Although the fall 2013 emissions were likely triggered by the sudden increase in soil moisture content on DOY 268 and 269, the total emissions remained low compared to snowmelt-induced emissions, indicating that the largest risk for N_2O losses from fall-applied urea lies in snowmelt events.

The generally higher N_2O emission rates from spring-applied compared to fall-applied fertilizers were likely the result of higher average temperatures and increased soil moisture content due to snowmelt. However, the maximum fluxes were not as high as from fall-applied fertilizers during snowmelt, likely because the majority of snowmelt-induced soil moisture had already infiltrated into the soil by the time fertilizer was applied.

Ammonia emissions from fall-applied fertilizers showed a trend opposite to N_2O emissions, as the majority of NH_3 emissions in the fall of 2012 and 2013 occurred shortly after application. This was expected, as NH_3 emissions from urea application are known to peak within several days after fertilizer application (Sommer et al., 2004). Ammonia emissions are governed by soil and air temperature with increases in both resulting in increased emissions (Sommer et al., 2004; Engel et al., 2011), which explains why NH_3 emissions in the earlier fall measurement period of 2013 resulted in more high-emission days than the later and therefore colder fall measurement period of 2012.

A large precipitation event at the end of the fall 2012 measurement period (25.6 mm on DOY 291) as well as the water provided through snowmelt in the spring of 2013 likely helped urea move into the soil, thereby reducing NH_3 emissions from fall-applied fertilizers after snowmelt. Dawar et al. (2011a) demonstrated how infiltrating water can move remaining urea into the soil while diluting NH_4^+ present at the soil surface. Similarly, Sanz-Cobena et al. (2011) reported that simulated rainfall of only 3 mm immediately after spreading urea enhances NH_3 emissions, whereas emissions are reduced by up to 89% after addition of 7 to 14 mm of water. These authors suggested that the higher water application rates served to move urea into the soil, where it was protected from surface volatilization losses. In the current study, the amount of water provided by the precipitation event on DOY 291 as well as by snowmelt, was therefore sufficient to completely mitigate NH_3 volatilization losses.

Ammonia emissions from spring-applied fertilizers were strongly increased compared to emissions from fall-applied fertilizers. This was likely the result of increased average tempera-

tures, the presence of soil moisture, and the lack of precipitation. Hargrove (1988) demonstrated that the application of fertilizer granules to a wet soil, followed by a period of drying, can drastically increase NH_3 losses. Furthermore, Engel et al. (2011) showed that up to 44% of applied urea-N was lost through NH_3 volatilization after application to a wet soil surface with low ($\leq$ 5 mm) subsequent precipitation in Montana. On the other hand, when urea was applied to a dry surface and strong precipitation events ($\geq$ 18 mm) followed, these authors reported that losses declined to less than 10% of applied N. These findings are in agreement with the current study, where the application of fertilizers to a wet soil in the spring without precipitation caused NH_3 emissions two to eight times higher than in the fall of either 2012 or 2013.

4.6.2 Performance of stabilized fertilizers in reducing NH_3 and N_2O emissions

In this study, stabilized fertilizers from both fall and spring-applied treatments showed mixed results in their efficacy in reducing N_2O emissions but showed promising results in mitigating NH_3 losses. The latter was evident where soil conditions favored NH_3 emissions (i.e., at CR1 in the spring of 2013). Specifically, in 2013, NH_3 emissions at CR1 were smaller in the fall and spring when the DI was applied compared to urea alone. Whereas the UI also reduced emissions, these reductions were significant only following the spring application. Previous studies showed similar results in that NBTPT typically reduced NH_3 emissions (Turner et al., 2010; Soares et al., 2012; Singh et al., 2013). Furthermore, these studies often utilized higher N application rates than in the current study, indicating that the efficacy of urease inhibitors on reducing NH_3 emissions could be expected to increase on soils with an inherently higher potential for NH_3 losses. Under such conditions, fall application losses of NH_3 from urea might even be significantly reduced by applying NBTPT.

Although products containing nitrification inhibitors (DI and NI) caused generally lower N_2O emissions than urea alone, this effect was not strong enough to result in a statistically significant reduction in average daily emissions (see Table 4.5). During the strong emission event immediately after snowmelt (DOY 126), emissions from DI only showed a reduction compared to urea at sites CR1 and CR2, while at ABR emissions from DI increased. Other studies have also observed mixed results in the efficacy of the nitrification inhibitor DCD to mitigate N_2O losses. For example, Shoji et al. (2001) demonstrated the successful mitigation of N_2O losses by DCD under irrigation treatments, while Parkin and Hatfield (2010) reported that inhibitors such as DCD and nitrapyrin resulted in no significant reduction in N_2O emissions over a one year measurement period. Di and Cameron (2002) suggested that the low efficiencies in reducing N_2O emissions from DCD-containing fertilizers could be the result of the premature degradation of the inhibitor while N_2O losses were still occurring. These authors reported that countering the degradation ef-

fect by repeating the application of DCD (five to nine times) resulted in an 82% reduction of N_2O emissions by DCD.

In the current study, it was unexpected that NI showed a reduction in NH_3 emissions despite the fact that it does not contain any urease inhibitor. The nitrification inhibitor DCD has been shown to prevent the transformation of NH_4^+/NH_3 to NO_3^- and thereby rather increase the potential for NH_3 losses (Zaman et al., 2008; Soares et al., 2012; Zaman et al., 2013). However, the application rate used by those authors (i.e., 150 to 600 kg N ha^{-1}) exceeded that of the current study and thus likely resulted in an increased accumulation of NH_4^+/NH_3 at the soil surface. Under those conditions, the inhibition of nitrification would preserve ammoniacal N at the soil surface and promote NH_3 losses. Moreover, the application of liquid urine by Zaman et al. (2008) and Zaman et al. (2013) is likely to result in a more rapid hydrolysis of urea than the application of granular urea (Vlek and Carter, 1983; Sommer et al., 2004). Therefore, the application of DCD prior to urine application, such as by Zaman et al. (2013), might have resulted in establishing the inhibitory effect of DCD in the soil solution and lead to a stronger reduction in nitrification activity by the time urine was applied.

The reduction of NH_3 emissions by NI in the current study is likely the result of the differences in granule size, as fertilizers containing NI were larger in granule size and dissolved slower than any other product. Despite the presence of the nitrification inhibitor, the slowed dissolution may have been the main factor preventing a rapid accumulation of ammoniacal N at the soil surface with subsequent NH_3 losses. This was in agreement with Black et al. (1987b), who demonstrated the effect of slowed dissolution due to larger granule size on reducing NH_3 emissions.

4.6.3 Differences in the performance of stabilized fertilizers among field sites

In the current study, the field sites CR1 and CR2 were selected due to their difference in soil pH, under the assumption that this would affect the potential for NH_3 and N_2O losses. This assumption was confirmed by the increased NH_3 and N_2O emission patterns at site CR1 compared to site CR2 during both the spring and the fall of 2013 (Figs. 4.9 to 4.7), indicating that soil conditions more favorable for gaseous N emissions existed at CR1. Indeed, the higher soil pH at site CR1 (7.6) compared to CR2 (6.9) may have contributed to the increased NH_3 losses at CR1, as high soil pH is known to favor NH_3 losses by shifting the balance between NH_4^+ and NH_3 in the soil solution towards NH_3 (Sommer et al., 2004). Furthermore, the consistently higher soil water content at CR1 compared to CR2 during both the spring and the fall of 2013 (Table 4.4) may have enhanced hydrolysis of urea at site CR1 and therefore promoted NH_3 losses through providing larger concentrations of NH_4^+/NH_3 at the soil surface that were susceptible to nitrification and denitrification to N_2O. The strongly increased emissions at CR1 indicated that the slight increase

in soil moisture content at this site was sufficient to improve conditions for urea hydrolysis, and subsequent gaseous emissions of both NH_3 and N_2O.

The fertilizer products DI and UI showed an increased efficacy in reducing NH_3 losses on the high-pH site (i.e., CR1) compared to the low-pH site (i.e., CR2). However, management of sites CR1 and CR2 included the weed control practice of burning stubble in the early spring, as soon as possible after snowmelt, for three consecutive years, while sites ABR and CHL were never burned. Because organic matter input is an important source of urease to the soil, burning could have strongly reduced organic matter abundance in the soil surface at CR1 and CR2, affecting hydrolysis of urea at the soil surface. This might explain why overall emissions in the current study were at the lower end of values reported in other studies. Studies testing the effect of spring burning on soil properties either demonstrate an increase in soil urease activity (Ajwa et al., 1999) or no effect (Dick et al., 1988; Picone et al., 2003). Using a controlled laboratory study, Picone et al. (2003) reported that burning affects the first 2.5 cm of soil when temperatures are high enough to denaturize the urease enzyme. These authors also suggested that burning could impact the input of organic matter to the soil, therefore reducing the long-term activity of urease.

Results from the ABR and CHL sites contrasted those observed at the Carrot River (CR1 & CR2) sites. At both ABR and CHL, N_2O emission fluxes from the stabilized UI and DI products were strongly elevated and when compared to emissions from the urea were associated with a large amount of variability during the peak emission period. This was surprising as the DI was expected to lower N_2O emissions due to the presence of DCD, while the UI was not expected to have a strong effect on N_2O emissions. However, the trend towards higher daily emissions from both products at those sites could have have partly been the result of an indirect effect of urease inhibition on N_2O emissions. It is possible that the fertilizer products containing no urease inhibitors (i.e., urea and NI) were affected by a more rapid hydrolysis of urea, resulting in stronger NH_3 volatilization losses, ultimately reducing the amount of N available for denitrification. Those products that were protected against hydrolysis (i.e., UI and DI), on the other hand, may have provided more available N for denitrification. This is in contrast to Dawar et al. (2011b), who reported that granular urea in combination with the urease inhibitor NBTPT resulted in lower N_2O emissions than urea alone.

The findings of the current study indicated that the effect of NBTPT on daily N_2O emissions was strongly dependent on site conditions, but that average daily losses were not significantly affected. Furthermore, the nitrification inhibitor DCD within NI showed only small or no reductions in N_2O emissions and was often mirrored by UI, although the latter does not contain any nitrification inhibitor. Moreover, the products DI and UI often showed similar trends in N_2O emissions. This indicated that that DCD played a minor role in reducing N_2O emissions under the relatively dry soil conditions of the Boreal Transition Zone of Saskatchewan.

4.7 Conclusions

This study demonstrated how application timing can influence gaseous N losses. While spring application of fertilizers was shown capable of mitigating potential N_2O losses from snowmelt-induced denitrification, the potential for NH_3 emissions was strongly elevated. The stabilized fertilizers DI, NI, and UI were all efficient in reducing gaseous losses of both NH_3 and N_2O. However, depending on the site conditions, such as pH and urease activity, the effect of stabilized fertilizers is variable, as was observed at sites ABR and CHL. Fall application, on the other hand, strongly reduced the potential for gaseous NH_3 emissions. Application close to snowfall or strong precipitation events likely caused infiltration of urea, with snowmelt further reducing the potential for NH_3 losses. Generally, snowmelt induced N_2O emissions were not higher than from spring-applied fertilizers. Therefore, when fall-application is required due to crop requirements, management routine or inaccessibility of the field in the spring, the use of stabilized fertilizers might not reduce the potential of gaseous N losses to a degree that would justify the added cost, compared to urea alone. On the other hand, when spring fertilization is possible, the application of stabilized fertilizers might strongly decrease both NH_3 and N_2O losses.

5 FERTILIZER-INDUCED EMISSIONS OF AMMONIA UNDER CONTROLLED SOIL ENVIRONMENTAL CONDITIONS

5.1 Preface

The previous chapter demonstrated that ammonia (NH_3) was the dominant form of gaseous N lost following broadcast applications of urea during forage seed production, and that stabilized fertilizers were able to significantly reduce those losses at one of two sites (CR1). However, variations in soil pH and soil moisture content at the two sites meant that it was difficult to determine how environmental variables affected NH_3 losses. Small differences in soil pH, moisture content, or temperature can influence the potential to which stabilized fertilizers reduce NH_3 volatilization losses. Thus, this chapter further explores the effects of soil pH, moisture content, and temperature on the efficacy of stabilized fertilizers in reducing NH_3 losses. Soils from the field sites used in the previous chapter were collected and the efficacy of stabilized fertilizers in reducing NH_3 losses was assessed under controlled soil environmental conditions. Furthermore, the activity of the soil enzyme urease and its inhibition by stabilized fertilizers was assessed for the respective soils to assess whether stabilized fertilizers differ in their potential to reduce NH_3 losses in different soils.

5.2 Abstract

Ammonia (NH_3) volatilization from urea-based fertilizers is one of the most important N loss pathways associated with surface application, and more than 50% of applied N can be lost through this pathway (Sommer et al., 2004). This reduces fertilizer nitrogen use efficiency in management systems where the incorporation of urea—one commonly used method for mitigating volatilization losses—is neither desired nor possible, such as in perennial forage seed production in Saskatchewan.

Recently, the use of urea-based stabilized fertilizers has shown promising results in mitigating NH_3 volatilization losses. Stabilized fertilizers contain either urease or nitrification inhibitors, or

both, and their use is aimed at blocking key processes in the N-cycle that contribute to N losses (i.e., urea hydrolysis and nitrification). For example, inhibiting soil urease prevents urea from being transformed to NH_3 and subsequently lost via volatilization, therefore allowing for precipitation events to move the urea into the soil, where the risk for volatilization losses is strongly reduced. Nitrification inhibitors, on the other hand, are often used to mitigate gaseous N losses associated with nitrification and dentrification (i.e., N_2O), but their use together with urease inhibitors, as is the case with some stabilized fertilizers, may increase NH_3 volatililzation losses under some conditions.

Successful in mitigation of gaseous NH_3 losses using stabilized fertilizers depends on the NH_3 volatilization potential of the soil, which in turn is governed by soil properties, such as soil pH, soil moisture content, soil temperature, and urease activity of the soil. While the effects of these soil properties on NH_3 volatilization losses have been well documented (Sherlock and Goh, 1984; Sommer et al., 2004), little is known about how well stabilized fertilizers will perform under different soil conditions.

The aim of this study was to assess the efficacy of surface-applied stabilized fertilizers in reducing NH_3 volatilization under controlled conditions. Soils from forage seed production fields in Saskatchewan were collected, and a series of bench-scale experiments in which the soil pH, moisture content, and temperature were manipulated to assess the efficacy of stabilized fertilizers in reducing NH_3 volatilization losses was conducted.

The stabilized fertilizer product containing a double inhibitor (DI) consistently reduced NH_3 volatilization losses across a variety of soil conditions, whereas the fertilizer product containing a nitrification inhibitor only (NI) did not reduce NH_3 losses. Differences in soil pH affected NH_3 losses from stabilized fertilizers in only one soil, indicating that soil pH was a secondary factor governing NH_3 emissions. Soil moisture content affected the magnitude of NH_3 losses and the composition of residual N (i.e., NH_4^+/NO_3^-), likely by delaying urea hydrolysis at low moisture levels and by enhancing dilution of urea at high moisture levels. Low soil temperature delayed the onset of NH_3 volatilization, likely as a result of slowed urea hydrolysis. Urease activity varied strongly between soils and was likely one main driver for NH_3 losses. A urea hydrolysis assay utilizing the stabilized fertilizer products indicated that the efficacy of those products in reducing NH_3 losses may be greater in soils with a high urease activity.

5.3 Introduction

Urea has become the most commonly used nitrogen (N) fertilizer in the world and is used more than all other synthetic N fertilizers together (Roy and Hammond, 2004; Glibert et al., 2006; IFA, 2014). Reasons for its popularity lie in the fact that urea has a high N content, is cost-effective,

and unlike ammonium nitrate, it is not explosive—making it safe to transport and store (Sommer et al., 2004).

Unfortunately, urea is prone to volatilization losses of ammonia (NH_3) when applied to the soil surface, which is one of the major reasons for the inefficiency associated with this fertilizer type. Volatilization losses can range from 0 to more than 50% of applied N (Sommer et al., 2004) and are one of the reasons why global N-use efficiency (NUE) is generally low. Raun and Johnson (1999) estimated that the global NUE in cereal production is about 33%, and in temperate regions NUE is generally lower than 70% (Malhi et al., 2001). Volatilization losses not only reduce NUE, but also pose a risk to the environment. For example, volatilized NH_3 can be transported away from the source and deposited into adjacent ecosystems, resulting in eutrophication and acidification (Schulze et al., 1989; Sommer and Hutchings, 1995; Asman et al., 1998; Sommer et al., 2004).

Farmers usually try to minimize volatilization losses by banding or incorporating the fertilizer into the soil (Sommer et al., 2004). This removes the urea from the soil:atmosphere interface and reduces the amount of ammoniacal N (NH_3 + NH_4^+) at the soil surface, thereby reducing the potential for volatilization. If incorporation of the fertilizer is neither possible nor desirable, farmers often try to broadcast the fertilizer shortly before a rainfall event to help move the urea into the soil and reduce potential volatilization losses.

Under management systems where no alternative to surface application of urea is available, such as in seed production of perennial forage grasses, other means of mitigating these losses are desired. One promising strategy to mitigate volatilization losses is the application of urea-based stabilized fertilizers that contain urease inhibitors, such as N-(n-butyl) thiophosphoric triamide or N-(2-nitrophenyl) phosphoric triamide. These urease inhibitors block the activity of the ubiquitous soil enzyme urease, which is responsible for the hydrolysis of urea to ammoniacal N in soils (Sommer et al., 2004; Trenkel, 2010). This prevents a majority of the applied N from being lost through volatilization, as urea cannot be volatilized before it has been hydrolyzed by soil urease.

There have been promising results from using stabilized fertilizers containing urease inhibitors, with a considerable amount of research conducted in New Zealand (Zaman et al., 2008, 2009; Saggar et al., 2013a,b; Singh et al., 2013; Zaman et al., 2013). These studies focused mainly on the application of urease inhibitors with cow urine. Under high N rates during simulated cow urination events (200 to 600 kg N ha^{-1}), urease inhibitors were able to reduce NH_3 volatilization losses by 22 to 93%. Similar results have been reported from surface applied urea granules in the United Kingdom (Sanz-Cobena et al., 2011), Germany (Ni et al., 2014), Brazil (Soares et al., 2012), Spain (San Francisco et al., 2011), and the United States (Engel et al., 2011; Frame et al., 2012).

Urease inhibitors are often applied together with nitrification inhibitors, such as dicyandiamide (DCD), to also suppress the formation of N_2O and leaching of NO_3^- (Zaman et al., 2008;

Trenkel, 2010; Soares et al., 2012; Ni et al., 2014). In some cases, however, nitrification inhibitors can increase potential NH_3 losses by preventingnitrification-induced reductions in the concentration of ammoniacal N at the soil surface (Zaman et al., 2009; Soares et al., 2012; Zaman et al., 2013). Because of such interactions between inhibitor types, it is important to test both urease and nitrification inhibitors together when assessing their efficacy to reduce NH_3 losses.

At present, it remains difficult to predict whether the use of stabilized fertilizers will reduce NH_3 losses from a given soil, as each soil may differ in the volatilization loss potential. The NH_3 losses from a soil are dependent on the physical, chemical, and biological properties of the soil, including soil pH, moisture content, and temperature (Sommer et al., 2004). The general relationship between these factors and NH_3 concentration at the soil surface is well documented (Sherlock and Goh, 1984; Sommer et al., 2004), though how changes in these factors affect the efficacy of stabilized fertilizers in reducing volatilization losses is less well understood. Because the use of stabilized fertilizers represents an added production cost to the farmer, there is a very practical need to better understand how well these products perform under different soil conditions.

The aim of this study was to assess how differences in soil pH, water content, and temperature affect the efficacy of stabilized fertilizers to reduce NH_3 volatilization from soils. Soil pH, water content, and temperature were manipulated under controlled conditions in the laboratory to determine how changes in these factors affect NH_3 volatilization from several, commercially available urea-based stabilized fertilizer products.

5.4 Materials and Methods

5.4.1 Soil characterization

Soils were collected from existing forage seed fields located near the towns of Carrot River and Arborfield, Saskatchewan. Two soils (CR1 and CR2) were selected from the Carrot River site; both were classified as Gleyed Dark Gray Chernozems of the Gronlid-Carrot River association (Saskatchewan Land Resource Centre, 1997b), formed on a mixture of loamy lacustrine and sandy fluvial materials, but they differed in soil organic matter content, pH and plant available P and S. The texture of the Carrot River soil was classified as very fine to fine sandy loam. Soil from the Arborfield site (ABR) was classified as a Dark Grey Chernozem of the Melfort-Tisdale association, formed on clayey lacustrine materials (Saskatchewan Land Resource Centre, 1997a). The surface texture of the ABR soil was characterized as a silty clay to silty clay loam.

Soil pH was measured on sieved and air-dried soil using a 1:10 (w/v) soil:0.01 M $CaCl_2$ suspension (Hendershot et al., 2007). Soils from the CR1 and CR2 varied in pH (7.8 and 6.9, respectively), whereas soil pH of the ABR soil was much lower (5.9). The gravimetric soil water content (GSWC) at field capacity for each soil was determined following the procedure described

byReynolds and Clarke Topp (2007) after application of -34000 Pa (4.95 PSI) to soil cores using a pressure plate apparatus. Soil characteristics are summarized in Table 5.1.

Table 5.1. Basic soil chemical and physical characteristics of soils used in incubation experiments.

Soil	GSWC at field capacity†	pH	Total C	Total N	C:N ratio	Urease activity‡
	g g^{-1}		— g kg^{-1} —			μg NH_4^+-N g^{-1} 2 h^{-1}
CR1	0.54	7.75	44.6	4.3	10.38	5.49 ± 1.43
CR2	0.49	6.87	29.5	3.2	9.21	6.65 ± 3.77
ABR	0.31	5.86	62.0	5.9	10.51	79.08 ± 4.99

† Gravimetric soil water content, expressed as g H_2O per g soil.

‡ Soil urease activity was measured according to Kandeler and Gerber (1988) on sieved soil (< 2 mm) at a gravimetric soil water content of 75% of field capacity.

5.4.2 Experimental set-up and design

Ammonia volatilization from soils amended with urea or a urea-based stabilized fertilizers containing a urease and/or nitrification inhibitor (Table 5.2) was assessed under controlled environment (i.e., soil water content and temperature) conditions using a modified version of the bench-scale system described by Woodward et al. (2011). Modifications included the use of 1-L Mason jars (sealed using No. 13 neoprene stoppers) as the soil reactors; replacing the rubber heating strips with water-resistant heating cables (designed for reptile enclosures) and insulating the inside of the chambers with aluminum bubble-wrap; and using larger (200 mL) acid traps filled with 0.01 *M* H_2SO_4. The entire system consisted of three temperature-controlled, bench-top cabinets (Figs. 5.1 and 5.2), each of which housed six independently plumbed soil reactors (Fig. 5.1g). Room temperature air entered the chamber after passing through an external humidifier (Fig. 5.1e) and then through an internal humidifier (Fig. 5.1f) maintained at a set temperature (i.e., 5, 15 or 26°C). Air exiting the soil reactors was directed into the acid traps (Fig. 5.1h) using vinyl tubing attached to an air stone to increase bubble size (i.e., maximize surface area) and then passed through a 11.5-cm column containing 200 mL 0.01 *M* H_2SO_4. Space limitations in the controlled environment room meant that only three volatilization cabinets could be operated at one time; thus necessitating multiple (n = 8) experimental runs to accommodate the various combinations of soil and environmental conditions. Treatment combinations (soil × water content × temperature) are presented in Table 5.3.

Table 5.2. Composition of the urea-based stabilized fertilizer products.

Product. ID	Product	Inhibitor type	Active ingredient†	Mode of application‡
C	- - -	- - -	- - -	- - -
U	Urea	- - -	- - -	- - -
UI-1	Agrotain®	Urease inhibitor	NBTPT	Surface coated§
UI-2	Piazur®	Urease inhibitor	2-NPT	Incorporated
NI	Alzon®	Nitrification inhibitor	DCD + TZ	Incorporated
DI	SuperU™	Dual inhibitor	NBTPT + DCD	Incorporated

† NBTPT = N-(n-butyl) thiophosphoric triamide; 2-NPT = N-(2-nitrophenyl) phosphoric triamide, DCD = dicyandiamide; TZ = 1H-1,2,4-triazole.

‡ The inhibitors were either incorporated into the fertilizer granules by the manufacturer during fertilizer production or coated onto the surface of the urea granules.

§ The product Agrotain® was coated to the urea granules at the recommended rate of 1.5 g kg^{-1} urea.

Table 5.3. Treatment combinations (soil × water content × temperature) during the volatilization experiments.

Experiment	Soil	Soil pH	GSWC (% FC)†	Temperature (°C)
1	CR1	7.75	75	26
2	CR2	6.87	75	26
3	ABR	5.86	75	26
4	ABR-L‡	7.07	75	26
5	ABR	5.86	100	26
6	ABR	5.86	50	26
7	ABR	5.86	75	5
8	ABR	5.86	75	15

† Gravimetric soil water content, expressed as a percentage of field capacity.

‡ A subsample of the Arborfield soil was limed with $CaCO_3$ to increase the pH to 7.07.

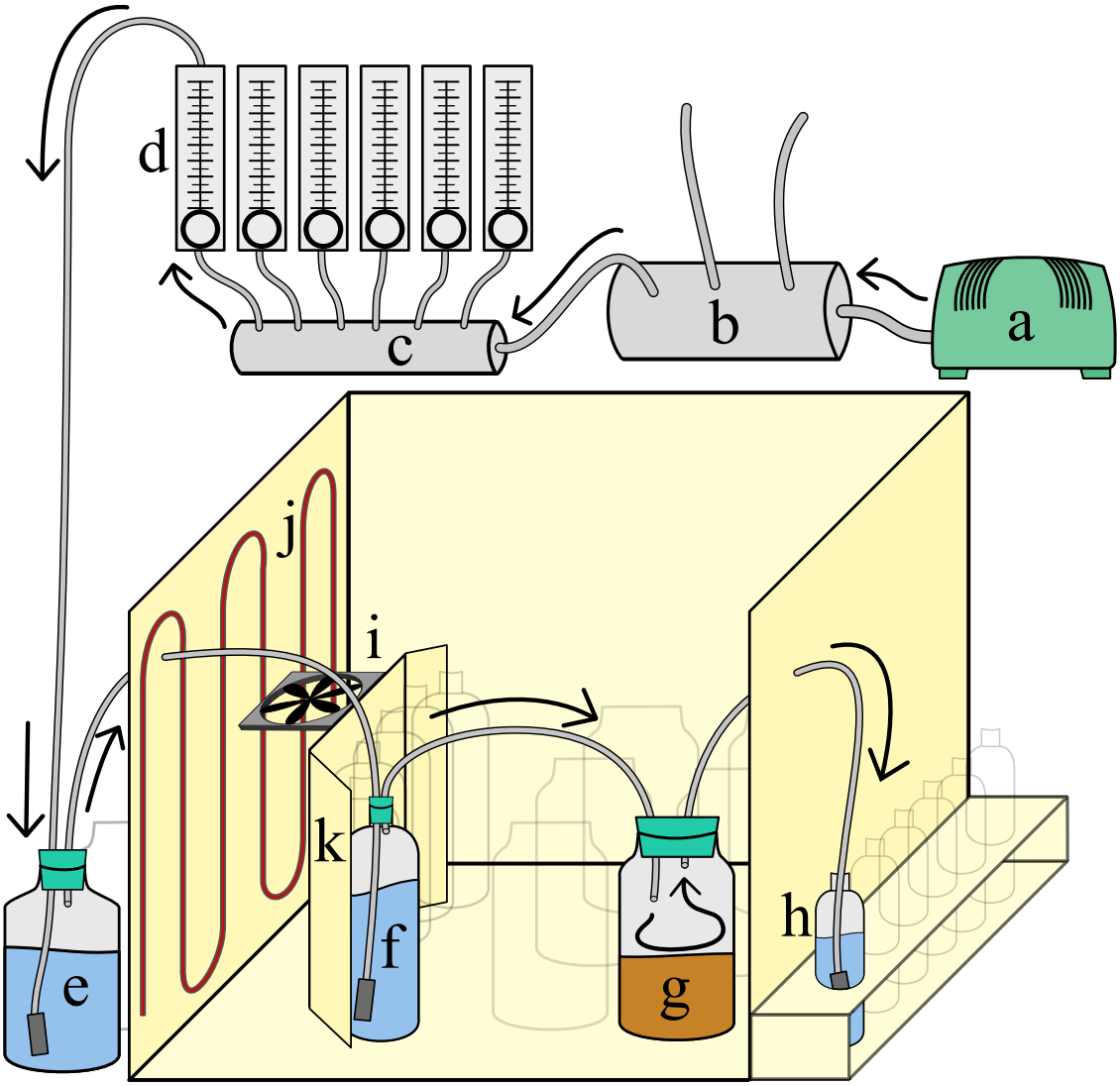


Fig. 5.1. Schematic of the modified wooden volatilization cabinets for measuring ammonia volatilization in the lab: (a) an air pump, (b) a large manifold distributing the air into (c) three small manifolds, (d) six air flow meters, (e) six external humidifiers, (f) six internal humidifiers, (g) six soil reaction chambers, (h) six acid traps, (i) a fan, (j) a heating cable, and (k) an air-guide baffle.

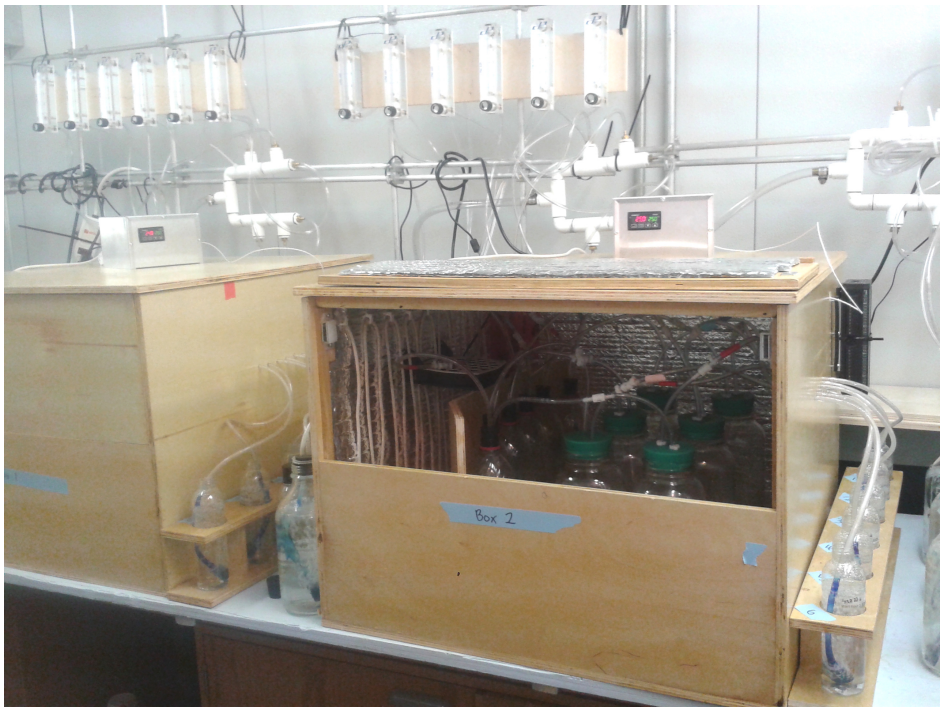


Fig. 5.2. Wooden cabinets for measuring ammonia volatilization in the lab.

Each experimental run was set up in a randomized complete block design (RCBD) with a single soil—amended with all five urea products (applied at a rate of 18.64 mg N per soil reactor), plus an unamended control (C) soil (see Table 5.2)—replicated three times and maintained at a constant temperature and soil water content. Note: each experimental run was blocked such that all six fertilizer treatments were included in each of the three volatilization cabinets—with each cabinet treated as a single replicate. Initially, NH_3 volatilization from the three field soils (plus a $CaCO_3$-amended ABR soil) was assessed under warm (26°C), moist (75% field capacity) conditions. Thereafter, the effects of temperature [at a constant (75%) soil water content] and soil water content [at a constant (26°C) temperature] on NH_3 volatilization were determined using only the ABR soil.

Prior to the start of each experimental run, 300 g of soil (air dried and screened to pass a 2-mm sieve) was weighed into each of 21 soil reactors (packed to a BD of 1.03 g cm^{-3} for CR1 and 0.80 g cm^{-3} for ABR soil) and the soil water content increased to the desired level by slowly adding the required amount of water to the soil surface. This technique was used to simulate a rainfall event while minimizing disturbance at the soil surface in order to maintain similar surface conditions for urea hydrolysis in each of the soil reactors. The low bulk density of both soils within the reaction chambers resulted in a rapid infiltration of the water with a homogeneous distribution throughout the soil. The reactors were then closed and pre-incubated at room temperature for

7 d. After pre-incubation, three reactors were randomly selected for destructive sampling and the soils extracted with 2 *M* KCl for the determination of available N (i.e., NO_3^- + NH_4^+/NH_3). The remaining 18 reactors were randomly divided between the three volatilization cabinets and the soil reactors connected to the air inlet and outlet lines—with the outlet lines connected to the acid traps and the system running to flush any gaseous NH_3 from the headspace of the reactors. After flushing the reactors for 1 h, the reactors were opened and the fertilizer granules applied to the surfaces of the soil. The reactors were then closed, the outlet lines connected to a new set of acid traps, and the airflow, maintained at 1 L min^{-1}, restarted.

The acid traps were replaced every 3 h during the first 24 h after application of the fertilizer treatments, then at 24- to 48-h intervals for another 12 to 13 d. At each change of the acid traps, the bottles containing the acid solution were sealed and stored at 4°C for up to 7 d until they were analyzed. Upon completion of the experimental run, the soils were sampled and extracted with 2 *M* KCl (20 g soil extracted with 200 mL KCl) to determine the amount of inorganic N (NO_3^- and NH_4^+/NH_3) in the soils. The NH_4^+ concentration in the acid traps, as well as the NO_3^- and NH_4^+ in the KCl extracts, were determined using a SmartChem® 200 Discrete Wet Chemistry Analyzer (Westco Scientific Instruments Inc.; Brookfield, CT, USA) as per the manufacturer's instructions.

5.4.3 Assessing native soil urease activity

Because the amount of urease present in the soil may influence the effectiveness of a stabilized fertilizer product containing a urease inhibitor, the urease activity in the Carrot River and Arborfield soils was assayed using the method described by Kandeler and Gerber (1988). This method measures the NH_4^+ released following incubation of the soil with an aqueous solution of urea. The soils were first pre-incubated at 75% field capacity and room temperature as described in the previous section, to prevent an increase in urea hydrolysis upon the addition of water to previously air-dried soil (Kandeler and Gerber, 1988). A 5-g subsample of the moist soil was then placed in a 50-mL Falcon tube to which 2.5 mL of a 0.08 *M* urea solution (4.8 g urea L^{-1}) was added, and the soils incubated at 37°C for 2 h. To account for background levels of NH_4^+ in the soils, the assay included a series of control soils, replicated four times, in which the urea solution was replaced with deionized water. Each soil was replicated four times. Following the 2-h incubation, 50 mL of a 1 *M* KCl–0.01 *M* HCl solution was added to each Falcon tube and the tubes placed on a mechanical shaker for 30 min; the resulting suspensions were then filtered and stored at 4°C in a refrigerator overnight to await analysis. The extracts were then analyzed for NH_4^+ using the SmartChem® 200 Discrete Wet Chemistry Analyzer (Westco Scientific Instruments Inc.; Brookfield, CT, USA) and the NH_4^+ release catalyzed by the native soil urease expressed as μg NH_4^+-N g $soil^{-1}$ 2 h^{-1}.

The urease assay also was used to determine how urea hydrolysis was affected by the four stabilized fertilizer products compared to urea alone. Following pre-incubation of the soils, 2.5 mL of fertilizer solution containing 4.8 g product L^{-1} were added, replicated four times. The soils were then incubated, extracted and analyzed as described above. In total, urea hydrolysis was assessed for 96 samples (four soils $\times$ six treatments $\times$ four replicates).

5.4.4 Calculations and statistical analyses

All calculations and statistical analyses were carried out in "R" (Version 3.0.2) (R Foundation for Statistical Computing, 2014). The mass of volatilized N contained in the acid traps was determined by multiplying the NH_4^+ concentration (μg N mL^{-1}) by the volume (200 mL) of the acid traps; cumulative volatilization losses (% applied N) were calculated by summing the mass of volatilized N in each trap and dividing it by the mass of applied N. Preliminary data analysis included assessing both the normality of the data and the homogeneity of the variance using the Shapiro Wilk's and Levene's test, respectively. A two-way analysis of variance (ANOVA) with fertilizer product and either soil pH, soil temperature, or soil moisture content as the factors was conducted on cumulative NH_3 emissions. When a significant interaction effect was detected, a univariate ANOVA was conducted on cumulative emissions for each experiment. Significant differences were tested post-hoc using Tukey's HSD test.

5.5 Results

5.5.1 Ammonia volatilization as affected by soil and pH

The Carrot River site from which the CR soils used in this study were collected, consisted of two areas with soils developed from the same parent materials and under the same management, but differed in pH and total N and C contents (Table 5.1). In general, regardless of the N source, NH_3 volatilization was negligible during the first 24 h following application of the fertilizer products, but then increased in a near-linear fashion over the next 96 h, before plateauing at 120 to 168 h after application (Fig. 5.3). For both the CR1 and CR2 soils, cumulative NH_3 volatilization (expressed as a percentage of the applied N) ranged from about 2% to 7% (Table 5.5)—with the lowest NH_3 losses associated with the stabilized fertilizer products containing a urease inhibitor (i.e., UI-1, UI-2 and DI).

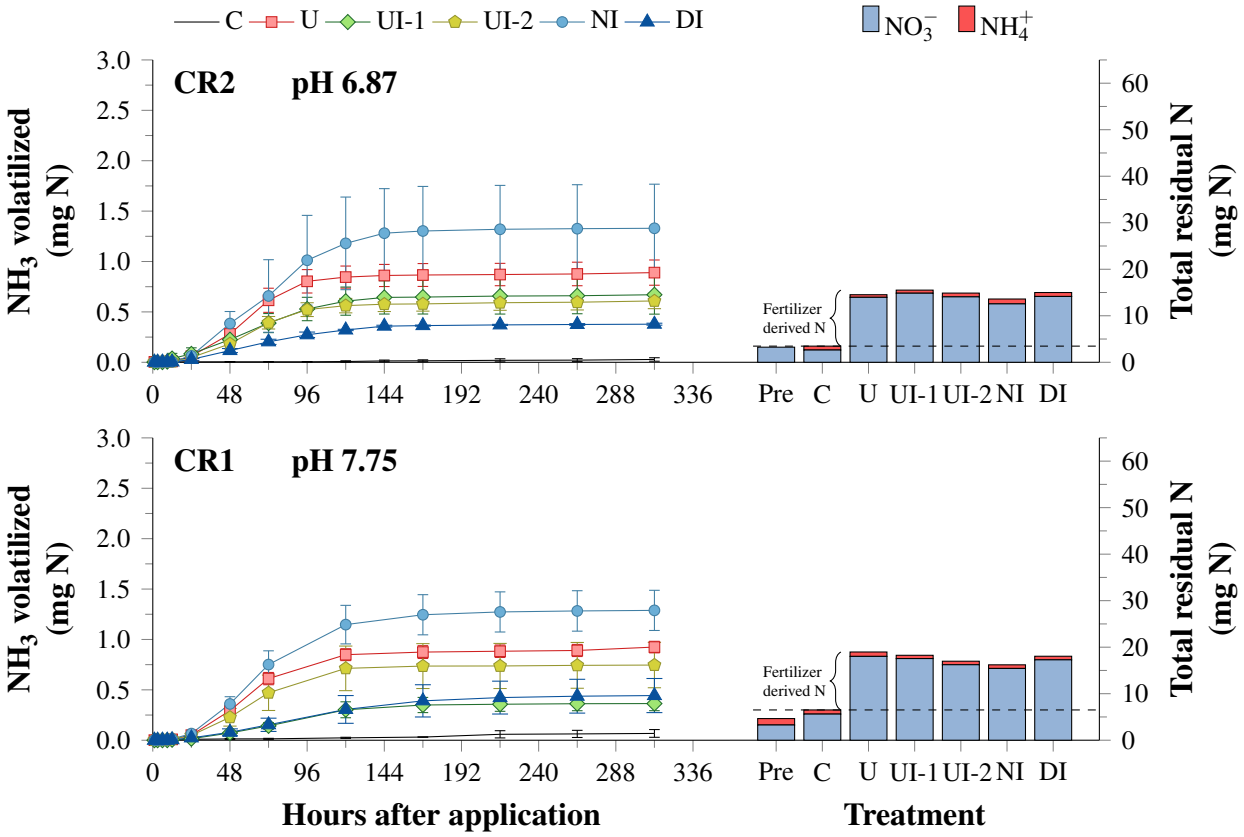


Fig. 5.3. Ammonia volatilization losses and residual N at different pH levels in Carrot River soil (CR1 and CR2) after application of different stabilized fertilizers. Error bars represent standard error of the mean. Pre = unfertilized soil at the beginning of the volatilization experiment, C = unfertilized control, U = untreated urea, UI-1 = urease inhibitor (NBTPT), UI-2 = urease inhibitor (2-NPT), NI = nitrification inhibitor (DCD + TZ), DI = double inhibitor (NBTPT + DCD).

Table 5.4. Cumulative NH_3 volatilization losses from soils differing in pH. Ammonia losses were measured following application of the different stabilized fertilizers, and are expressed as either mg N or as a percentage of applied N. Different lowercase letters indicate significant differences between means groups at $P < 0.05$.

		Fertilizer product‡					
Soil†	**pH**	**U**	**UI-1**	**UI-2**	**NI**	**DI**	**Mean**
		mg N					
CR2	6.87	0.89 ± 0.13	0.67 ± 0.19	0.61 ± 0.08	1.33 ± 0.44	0.38 ± 0.01	0.77 a
CR1	7.75	0.92 ± 0.06	0.36 ± 0.07	0.75 ± 0.23	1.29 ± 0.20	0.44 ± 0.17	0.75 a
Mean		0.91 b	0.52 c	0.68 bc	1.31 a	0.41 c	
		% applied N					
CR2	6.87	4.72 ± 0.73	3.56 ± 0.97	3.24 ± 0.36	7.03 ± 2.39	2.00 ± 0.05	4.11 a
CR1	7.75	4.87 ± 0.32	1.96 ± 0.37	3.90 ± 1.22	6.74 ± 1.06	2.32 ± 0.90	3.95 a
Mean		4.80 b	2.76 c	3.57 bc	6.89 a	2.16 c	

† CR1/CR2= Carrot River soil, ABR = Arborfield soil, ABR-L = limed Arborfield soil.

‡ U = untreated urea, UI-1 = urease inhibitor (NBTPT), UI-2 = urease inhibitor (2-NPT), NI = nitrification inhibitor (DCD + TZ), DI = double inhibitor (NBTPT + DCD).

Soil pH appeared to have no significant effect on NH_3 volatilization from the urea (U) fertilizer, with cumulative losses ranging from 4.7% to 4.9% of applied N in the CR1 and CR2 soils, respectively. However, NH_3 volatilization rates and cumulative NH_3 losses associated with the DI product were significantly ($P = 0.027$) lower than those associated with the untreated U—with the dual inhibitors (NBTPT + DCD) reducing NH_3 volatilization by 52% and 57% in the CR1 and CR2 soils, respectively. Similar results were obtained with the UI-1 product (i.e., urea + NBTPT), but only in the more alkaline CR1 soil. At the same time, NH_3 losses associated with the UI-2 product (urea + 2-NPT) did not differ from those associated with the untreated U in either soil. Cumulative NH_3 losses associated with the NI product (urea + DCD) were generally greater than those associated with the U or UI products (Fig. 5.3), but were also more variable; consequently, differences between the U and NI were not significant ($P = 0.107$). In both soils, however, NH_3 losses from stabilized fertilizer products containing a urease inhibitor were significantly ($P = 0.015$) lower than those from the NI.

The amount of available N (i.e., NO_3^- + NH_4^+) remaining in the soils at the end of the incubations ranged from about 16 to 19 mg N, with the majority of the N (92 to 96%) present as NO_3^-

(Fig. 5.3). Moreover, the amount of available N present was independent of both the source of the N and soil (see Table A.8)

To further assess the impact of soil pH on the ability of the stabilized fertilizer products to reduce NH_3 losses, a subsample of the Arborfield soil (ABR; pH 5.9) was limed (ABR-L) to a pH of 7.1 prior to pre-incubation and application of the urea and urea-based stabilized fertilizer products. Ammonia volatilization patterns showed clear differences between the native (ABR; Fig. 5.4a) and limed (ABR-L; Fig. 5.4b) soils—with a shorter lag period and higher rates of volatilization from untreated urea in the low pH ABR soil. However, there was a significant interaction effect between fertilizer product and soil pH (see Table A.5), thus a univariate ANOVA was conducted on each soil separately. Cumulative NH_3 losses from untreated urea from the Arborfield soil were numerically greater at pH 5.9 (Fig. 5.4a) than at pH 7.1 (Fig. 5.4b); however, losses from UI-1 and UI-2 were numerically lower at the low-pH soil. Furthermore, when the N source was untreated urea (U), cumulative NH_3 losses from the ABR (22% of applied N) and ABR-L soil (14% of applied N) were 3- to 4.5-times greater than those from the Carrot River soils. However, as was the case with the Carrot River soils, NH_3 volatilization rates and cumulative NH_3 losses from Arborfield soils amended with the DI product were significantly ($P = 0.05$) lower than those associated with the untreated U—with the dual inhibitors reducing NH_3 volatilization by 66% and 72% in the ABR and ABR-L soils, respectively. Similar results were obtained with the UI-2 product, but only in the more acidic ABR soil. Likewise, cumulative NH_3 losses associated with the UI-1 product in the low pH ABR soil were numerically lower than those associated with U alone; however, because of the large variability associated with the U treatment (see Fig. 5.4a), these differences were not significant ($P = 0.335$). Concentrations of available soil N were numerically greater in the Arborfield soils (Fig. 5.4) than in the Carrot River soils (Fig. 5.3). Moreover, regardless of N source, available NH_4^+-N concentrations in the fertilized treatments (in excess to the unfertilized control) were much greater in the low pH ABR soil than in the limed ABR-L soil (see Table A.11). Although the stabilized fertilizer products had no effect on available N concentrations in the Carrot River soils, treatment effects were observed in the Arborfield soils. Indeed, NH_4^+ concentrations in the soils amended with the stabilized fertilizer product containing a double inhibitor (i.e., DI) were greater than those in soils amended with the untreated urea (U). Conversely, NO_3^- concentrations in the DI treated soils were numerically equal to or lower than those in the U treated soils.

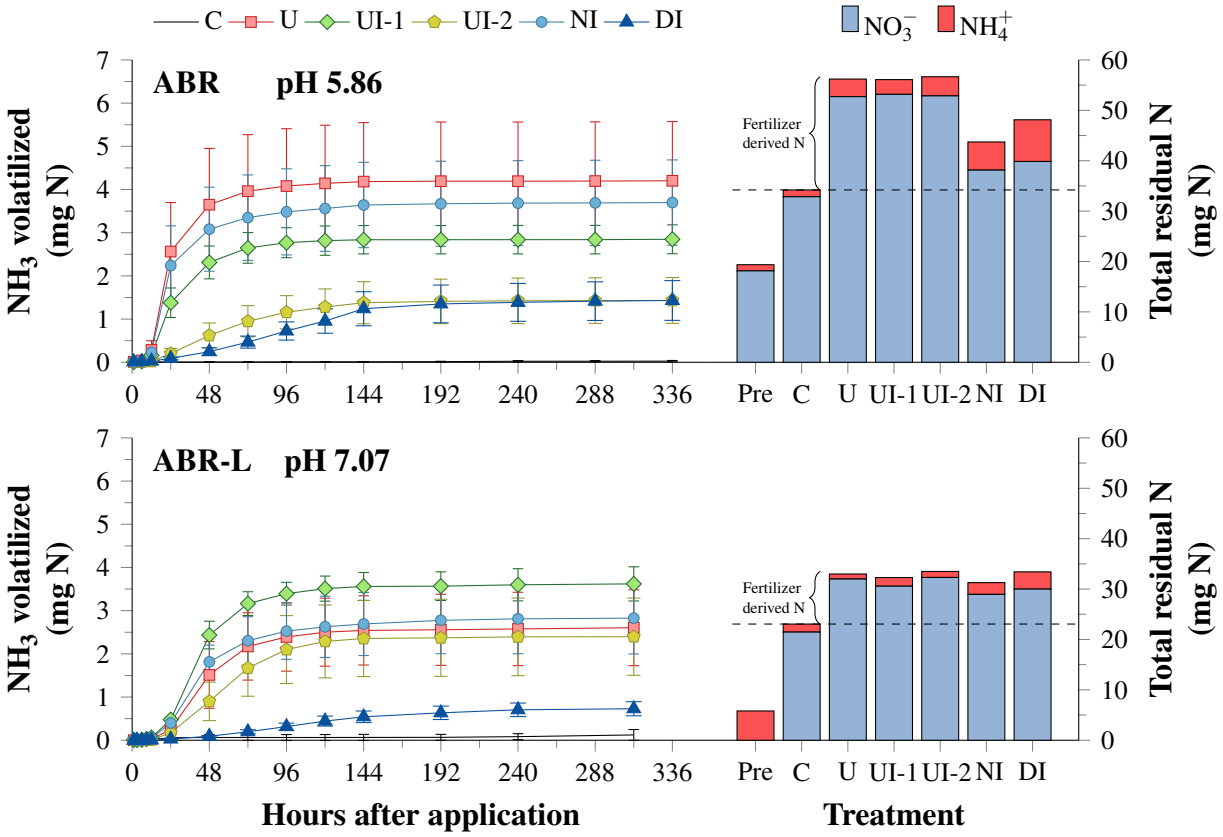


Fig. 5.4. Ammonia volatilization losses and residual N at different pH levels in the unamended (ABR) and limed (ABR-L) Arborfield soil after application of different stabilized fertilizers. Error bars represent standard error of the mean. Pre = unfertilized soil at the beginning of the volatilization experiment, C = unfertilized control, U = untreated urea, UI-1 = urease inhibitor (NBTPT), UI-2 = urease inhibitor (2-NPT), NI = nitrification inhibitor (DCD + TZ), DI = double inhibitor (NBTPT + DCD).

Table 5.5. Cumulative NH_3 volatilization losses from soils differing in pH. Ammonia losses were measured following application of the different stabilized fertilizers, and are expressed as either mg N or as a percentage of applied N. Within soils, different lowercase letters indicate significant differences at $P < 0.05$.

		Fertilizer product‡				
Soil†	**pH**	**U**	**UI-1**	**UI-2**	**NI**	**DI**
		mg N				
ABR	5.86	4.20 ± 1.37 a	2.85 ± 0.33 ab	1.43 ± 0.53 b	3.70 ± 0.99 a	1.43 ± 0.46 b
ABR-L	7.07	2.61 ± 0.88 a	3.62 ± 0.40 a	2.40 ± 0.89 ab	2.83 ± 0.83 a	0.73 ± 0.16 b
		% applied N				
ABR	5.86	22.24 ± 7.61 a	14.90 ± 1.80 ab	7.58 ± 2.80 b	19.67 ± 5.20 a	7.58 ± 2.33 b
ABR-L	7.07	13.88 ± 4.67 a	19.46 ± 2.06 a	12.93 ± 4.81 ab	15.16 ± 4.45 a	3.91 ± 0.87 b

† CR1/CR2= Carrot River soil, ABR = Arborfield soil, ABR-L = limed Arborfield soil.

‡ U = untreated urea, UI-1 = urease inhibitor (NBTPT), UI-2 = urease inhibitor (2-NPT), NI = nitrification inhibitor (DCD + TZ), DI = double inhibitor (NBTPT + DCD).

5.5.2 Ammonia volatilization as affected by soil moisture

The effect of soil moisture on NH_3 emissions from soil amended with the different stabilized fertilizer products was investigated using the Arborfield (ABR) soil. Experimental runs were carried out with the soils maintained at a constant temperature (26°C) and GSWC corresponding to 50, 75 or 100% of field capacity (FC). Ammonia volatilization curves for the urea and stabilized fertilizer products under the different soil water regimes are presented in Fig. 5.5. Ammonia volatilization from both the untreated U and stabilized fertilizer products was strongly influenced by soil water content (Table 5.6). For example, cumulative NH_3 volatilization from U (expressed as a percentage of applied N) increased from 10.7% at a soil water content corresponding to 50% FC to 22.2% at a soil water content corresponding to 75% FC (Table 5.6). Further increasing the soil water content to a value corresponding to 100% FC resulted in a large decrease in NH_3 volatilization, with cumulative NH_3-N losses of only 5.8%. Fertilizer effects were observed under all three water regimes, with the DI yielding significantly (P = 0.032) less NH_3 than U at 50 and 75% FC, and less than the NI at 100% FC (Table 5.6). Likewise, cumulative NH_3 emissions associated with the UI-2 were generally lower than those from U, though the difference was significant (P = 0.015) only at 75% FC.

Following the initial pre-incubation stage, available soil N was numerically greater at 75% FC (19.4 mg N) than at either 50% or 100% FC (6.0 and 10.7 mg N, respectively). Moreover, whereas NH_4^+ accounted for only 6% of the available N at 75% FC, it accounted for 100% of the available N at both 50% and 100% FC (Fig. 5.5). During the subsequent 14-d incubation, however, the amount of available N in the soils increased strongly at 100% FC relative to 50% FC—with NO_3^- making up the bulk of the increase (see Table A.14). Under drier conditions (i.e., at 50% FC), the increase was much smaller, though NO_3^- remained the dominant form of available N.

Relative to the unfertilized control (C) soil, addition of the urea and stabilized fertilizer products resulted in an increased supply of available N at the end of the 14-d incubation—with the largest increases occurring at 75% and 100% FC (Fig. 5.5). In general, the available soil N supply was impacted the least by the stabilized fertilizer products containing the nitrification inhibitor DCD (NI and DI). Conversely, fertilizer products that did not contain the nitrification inhibitor (i.e., U, UI-1, and UI-2) had the greatest impact on available soil N during the 14-d incubation. Moreover, with these products, the NH_4^+ concentration decreased with increasing soil moisture; indeed, the ratio of NH_4^+ to NO_3^- decreased 10-fold as soil moisture was increased from 50% to 100% FC. At the same time, regardless of soil water content, NH_4^+ concentrations were greatest in the soils amended with NI and DI (see Table A.14), and the ratio of NH_4^+ to NO_3^- in these soils decreased only about 3-fold as soil moisture was increased.

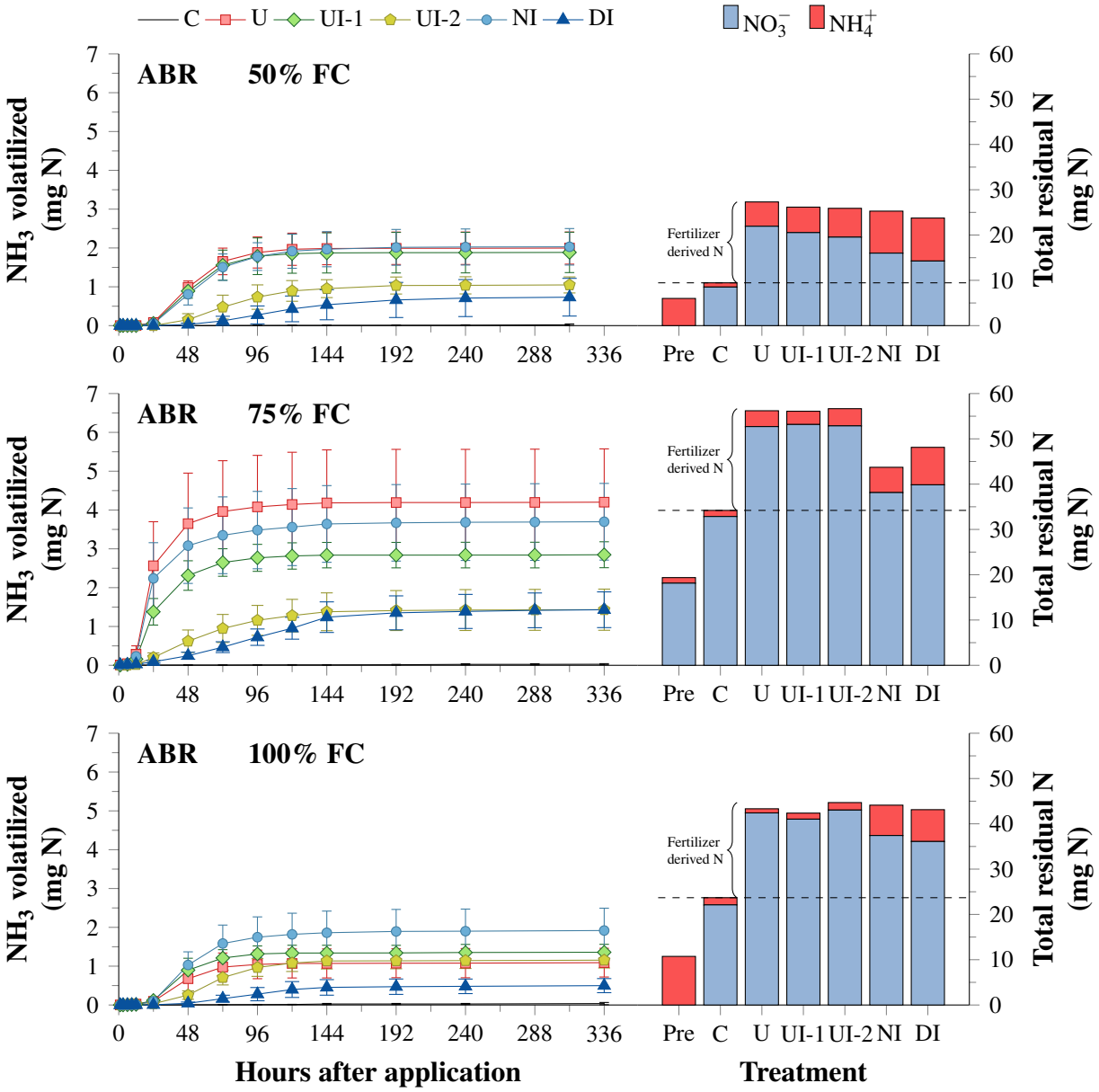


Fig. 5.5. Ammonia volatilization losses and residual N at different soil moisture conditions in Arborfield (ABR) soil after application of different stabilized fertilizers. Error bars represent standard error of the mean. Pre = unfertilized soil at the beginning of the volatilization experiment, C = unfertilized control, U = untreated urea, UI-1 = urease inhibitor (NBTPT), UI-2 = urease inhibitor (2-NPT), NI = nitrification inhibitor (DCD + TZ), DI = double inhibitor (NBTPT + DCD).

Table 5.6. Cumulative NH_3 volatilization losses at different soil moisture conditions. Ammonia losses were measured following application of the different stabilized fertilizers, and are expressed as either mg N or as a percentage of applied N. Different lowercase letters indicate significant differences between means groups at $P < 0.05$.

		Fertilizer product‡					
GSWC†		**U**	**UI-1**	**UI-2**	**NI**	**DI**	**Mean**
g g^{-1}	**% FC**	**mg N**					
0.15	50%	2.00 ± 0.41	1.89 ± 0.52	1.05 ± 0.21	2.03 ± 0.47	0.73 ± 0.49	1.54 b
0.23	75%	4.20 ± 1.37	2.85 ± 0.33	1.43 ± 0.53	3.70 ± 0.99	1.43 ± 0.46	2.72 a
0.31	100%	1.09 ± 0.38	1.36 ± 0.21	1.15 ± 0.20	1.91 ± 0.57	0.50 ± 0.19	1.20 b
Mean		2.43 a	2.03 ab	1.21 bc	2.55 a	0.89 c	
		% applied N					
0.15	50%	10.68 ± 2.20	10.11 ± 2.78	5.59 ± 1.13	10.85 ± 2.61	3.90 ± 2.55	8.23 b
0.23	75%	22.24 ± 7.61	14.90 ± 1.80	7.58 ± 2.80	19.67 ± 5.20	7.58 ± 2.33	14.39 a
0.31	100%	5.84 ± 1.97	7.30 ± 1.13	6.13 ± 1.07	10.21 ± 3.00	2.67 ± 1.00	6.43 b
Mean		12.92 a	10.77 ab	6.44 bc	13.58 a	4.72 c	

† Gravimetric soil water content, expressed as either g per g soil or as a percentage of field capacity.

‡ U = untreated urea, UI-1 = urease inhibitor (NBTPT), UI-2 = urease inhibitor (2-NPT), NI = nitrification inhibitor (DCD + TZ), DI = double inhibitor (NBTPT + DCD).

5.5.3 Ammonia volatilization as affected by soil temperature

The effect of soil temperature on NH_3 emissions from soil amended with the different stabilized fertilizer products also was investigated using the Arborfield (ABR) soil. Experimental runs were carried out with the soils maintained at a constant soil water content (corresponding to 75% of field capacity) and a soil temperature of 5, 15, or 26°C. Ammonia volatilization curves for the urea and stabilized fertilizer products at the different soil temperatures are presented in Fig. 5.6. Ammonia volatilization from the urea and stabilized fertilizer products exhibited a strong response to soil temperature—with a significant shortening of the lag period (i.e., the time leading up to the onset of NH_3 volatilization) as the soil temperature increased. Moreover, both the rate and magnitude of the NH_3 emissions increased as the soil temperature increased.

At 5°C, there was essentially no NH_3 volatilization from the U amended soils until about 120 h after the fertilizer application, and cumulative NH_3 losses from the U (expressed as a percentage of applied N) totaled only about 8% during the 14-d incubation (Table 5.7). Increasing the soil temperature to 15°C shortened the lag period to about 48 h and increased the rate of NH_3 volatilization, but had only a relatively small effect on cumulative emissions (i.e., cumulative emissions increased by only 1.4% relative to those at 5°C). Increasing the soil temperature to 26°C, further shortened the lag period to only 12 h and increased both the rate of and magnitude of the NH_3 emissions—with cumulative emissions at 26°C accounting for about 22% of the applied N. Similar trends were observed for the different stabilized fertilizer products (Fig. 5.6), with both the rate and magnitude of the emissions being greatest for the NI and the lowest for the DI at all three soil temperatures. In general, NH_3 volatilization associated with stabilized fertilizer products containing a urease inhibitor exceeded that from the dual inhibitor product (DI) but was lower than that from the product containing only a nitrification inhibitor (NI); however, this effect was significant only for UI-2 (see Table 5.7).

Soil temperature had a significant effect on the supply of available soil N (Fig. 5.6). The available soil N supply at the start of each experimental run (i.e., after the pre-incubation at room temperature and before application of the fertilizer products) was greater in the first experiment using ABR soil (i.e., at 26°C) than in any other experiment. Moreover, whereas NH_4^+ accounted for only 6% of the available N at 26°C, it accounted for 100% of the available N at both 5°C and 15°C (Fig. 5.6). The available soil N supply increased in the control (non-fertilized) soils during the subsequent 14-d incubation, with NO_3^- being the dominant form of available N at all three temperatures.

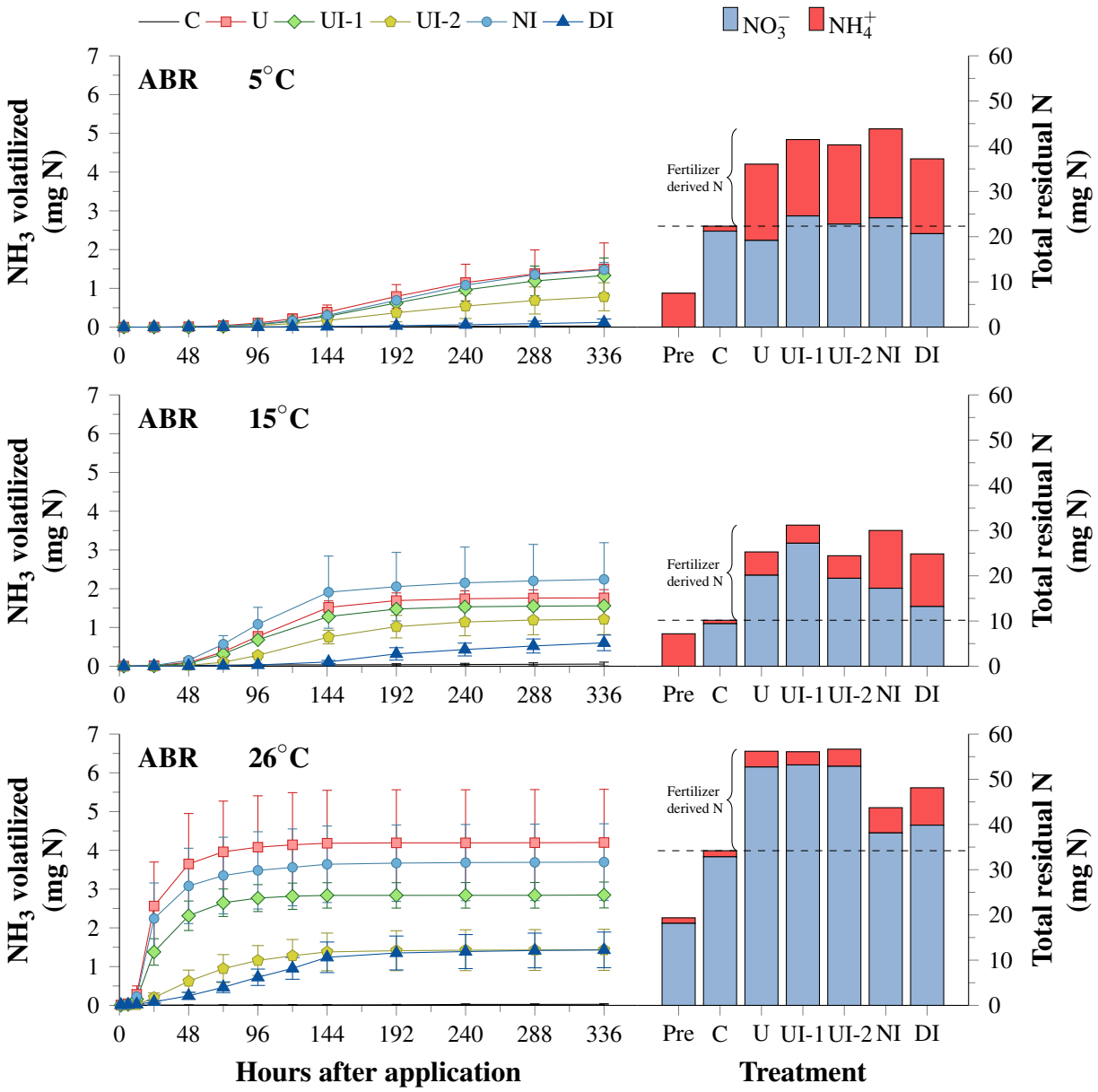


Fig. 5.6. Ammonia volatilization losses and residual N at different soil temperatures in Arborfield (ABR) soil after application of different stabilized fertilizers. Error bars represent standard error of the mean. Pre = unfertilized soil at the beginning of the volatilization experiment, C = unfertilized control, U = untreated urea, UI-1 = urease inhibitor (NBTPT), UI-2 = urease inhibitor (2-NPT), NI = nitrification inhibitor (DCD + TZ), DI = double inhibitor (NBTPT + DCD).

Table 5.7. Cumulative NH_3 volatilization losses at different soil temperature conditions. Ammonia losses were measured following application of the different stabilized fertilizers, and are expressed as either mg N or as a percentage of applied N. Different lowercase letters indicate significant differences between means groups at $P < 0.05$.

	Fertilizer product†					
Temperature	**U**	**UI-1**	**UI-2**	**NI**	**DI**	
°C	mg N					**Mean**
5	1.50 ± 0.67	1.33 ± 0.45	0.78 ± 0.36	1.48 ± 0.19	0.12 ± 0.09	1.04 c
15	1.76 ± 0.21	1.56 ± 0.08	1.21 ± 0.39	2.24 ± 0.95	0.60 ± 0.20	1.48 b
26	4.20 ± 1.37	2.85 ± 0.33	1.43 ± 0.53	3.70 ± 0.99	1.43 ± 0.46	2.72 a
Mean	2.49 a	1.91 ab	1.14 bc	2.47 a	0.72 c	
	% applied N					
5	8.04 ± 3.67	7.15 ± 2.33	4.17 ± 1.94	7.97 ± 1.07	0.66 ± 0.47	5.60 c
15	9.43 ± 1.21	8.37 ± 0.51	6.54 ± 2.14	11.98 ± 5.08	3.23 ± 1.07	7.91 b
26	22.24 ± 7.61	14.90 ± 1.80	7.58 ± 2.80	19.67 ± 5.20	7.58 ± 2.33	14.39 a
Mean	13.24 a	10.14 ab	6.10 bc	13.20 a	3.82 c	

† U = untreated urea, UI-1 = urease inhibitor (NBTPT), UI-2 = urease inhibitor (2-NPT), NI = nitrification inhibitor (DCD + TZ), DI = double inhibitor (NBTPT + DCD).

Relative to the unfertilized control, application of the urea and stabilized fertilizer products resulted in increases in total available N, though the distribution of NH_4^+ and NO_3^- in the soils varied with soil temperature (Fig. 5.6). There was a significant effect of soil temperature on soil NO_3^- ($P = 0.009$) and NH_4^+ ($P < 0.001$) (Table A.18 and A.19, respectively). For example, at 5°C, soil NH_4^+ from fertilizer products was significantly higher than at 15°C and 26°C (Table 5.7). Conversely, soil NO_3^- were lowest at 5°C compared to 15°C and 26°C (Table 5.7). Furthermore, at 5°C, there were no fertilizer effects on the available soil N supply and virtually all of the available N was present as NH_4^+ (Fig. 5.6a, Table 5.7). Increasing the soil temperature to 15°C resulted in an increase in the amount of soil NO_3^- present in the soils, as well as a concomitant decrease in the ratio of NH_4^+ to NO_3^-, for all the fertilizer products (Fig. 5.6b). Similar results were observed at 26°C (Fig. 5.6c).

5.5.4 Impact of stabilized fertilizer products on the rate of urea hydrolysis

Ammonium release catalyzed by native soil urease, and the effects of the stabilized fertilizer products on NH_4^+ release, were determined for the Carrot River and Arborfield soils over a 2-

h period, following addition of the fertilizer to the soil (Fig. 5.7). There were clear differences between the soils; e.g., significantly ($P < 0.001$) more NH_4^+ was released from the untreated urea (U) in the Arborfield soils (79 and 96 μg NH_4^+-N g soil^{-1} 2 h^{-1} for the ABR and ABR-L soils, respectively) than in the Carrot River soils (5.5 and 6.6 μg NH_4^+-N g soil^{-1} 2 h^{-1} for the CR1 and CR2 soils, respectively). Not surprisingly, the stabilized fertilizer products that contained a urease inhibitor (UI-1, UI-2 and DI) generally suppressed ($P < 0.001$) NH_4^+ release relative to the untreated urea and urea treated with a nitrification inhibitor alone (NI). Compared to the native soil (ABR), liming the Arborfield soil (ABR-L) increased the release of NH_4^+ from the untreated urea, but had little effect on NH_4^+ release from the stabilized fertilizer products. However, due to the enhanced NH_4^+ release from the untreated urea, the relative effect of the stabilized fertilizers was magnified in the ABR-L soil.

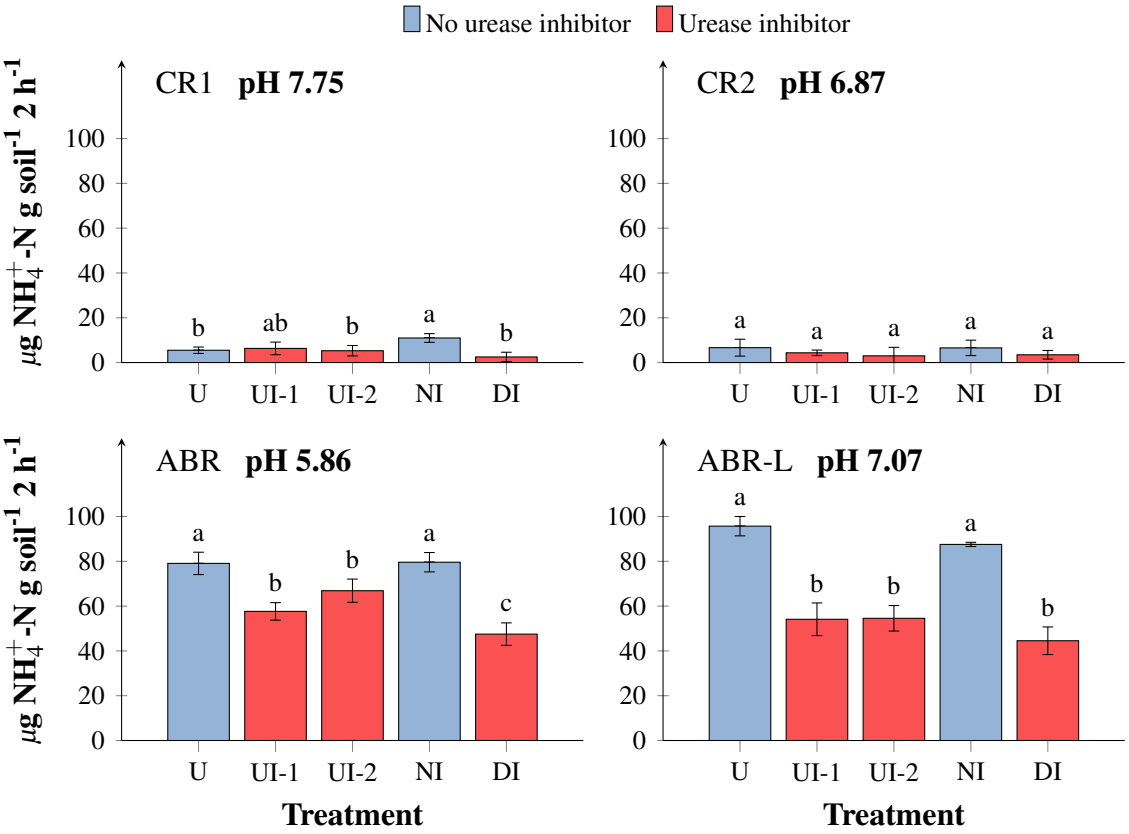


Fig. 5.7. Ammonium release catalyzed by urease in soil from Carrot River and Arborfield under different pH conditions, incubated for 2 h at 37°C. Error bars represent standard deviation of four replicates. Different lowercase letters indicate significant differences at $P < 0.05$.

5.6 Discussion

Ammonia volatilization losses were affected by the soil (i.e., Carrot River *vs.* Arborfield). Differences in soil pH showed a significant interaction with fertilizer treatment in the ABR soil (see Table A.5). This effect was not observed in the Carrot River soils (see Table A.4), likely because the difference in pH between CR1 and CR2 was not large enough to affect the equilibrium between NH_3 and NH_4^+ in the soil solution. As a result, onset and magnitude of cumulative NH_3 volatilization losses were similar in both CR1 and CR2 soils, with the stabilized fertilizers showing similar trends relative to untreated urea in both soils. Furthermore, soil from the ABR site was considerably lower in soil pH (5.86), compared to the CR1 and CR2 soils (i.e., 7.75 and 6.87, respectively), yet volatilization losses from the untreated urea were 4-fold greater in the ABR soil than in either the CR1 or CR2 soil. Moreover, liming of Arborfield soil (ABR-L) generally resulted in lower volatilization losses than from native Arborfield soil (ABR). This was unexpected, as NH_3 losses are known to generally increase with increasing soil pH (Sommer et al., 2004).

Ammonia losses observed in this study are in agreement with the model proposed by Sherlock and Goh (1984), cited by Sommer et al. (2004), which describes the relationship between concentration of aqueous NH_3 and the temperature and pH of an ammoniacal soil solution at the soil-air interface (See Eq. 2.3). According to this model, an increase in pH of the soil solution to > 8 can shift the equilibrium between NH_4^+ and NH_3 in the soil solution towards NH_3, and this effect is amplified when the temperature increases. However, because the pH of the Carrot River and Arborfield soils was < 8 and therefore not affecting the balance between NH_4^+ and NH_3, it is likely that the magnitude of NH_3 emissions was not entirely governed by the initial soil pH. However, the hydrolysis of urea has been shown to increase the pH in the immediate proximity to the urea granules, thus increasing NH_3 losses even from soils low in pH Sommer et al. (2004). A more rapid hydrolysis of urea within the Arborfield soil compared to the Carrot River soil could have been the reason why NH_3 losses were generally higher in Arborfield soil, despite the lower soil pH.

The magnitude to which soil pH and temperature affect the concentration of NH_3 in the soil—and therefore the potential for volatilization losses—is dependent on the total ammoniacal N (TAN) concentration of the soil solution (Eq. 2.3). When the moisture content is high, it is more likely that the urea-N will be dissolved and transported away from the source, thus reducing both the concentration of TAN as well as the effect of urea hydrolysis on the pH at the soil surface. Under drier soil conditions, however, the urea-N will be less diluted, resulting in higher TAN concentrations and a stronger localized increase in soil pH (i.e., conditions favoring NH_3 volatilization).

This was observed in the current study, where the soil moisture content at 75% FC was higher in the CR1 and CR2 soils (0.41 and 0.37 g H_2O g $soil^{-1}$, respectively) than in the ABR soil (0.23 g H_2O g $soil^{-1}$). Urea hydrolysis was assumed to be complete and the soil water content was assumed

to be evenly distributed within the soil, thus the addition of 18.64 mg N would have resulted in a lower TAN concentration in the surface 1-cm of Carrot River soil (0.038 mol L^{-1}) than in the surface 1-cm of Arborfield soil (0.096 mol L^{-1}). Using these values—and assuming that urea hydrolysis increased soil pH adjacent to the granules to > 8—the model predicted concentrations of aqueous NH_3 in Arborfield soil that were more than twice as high as those in the Carrot River soil, at all temperatures (Fig. 5.8). In turn, this would suggest greater NH_3 volatilization from the Arborfield soils (Appendix A.1).

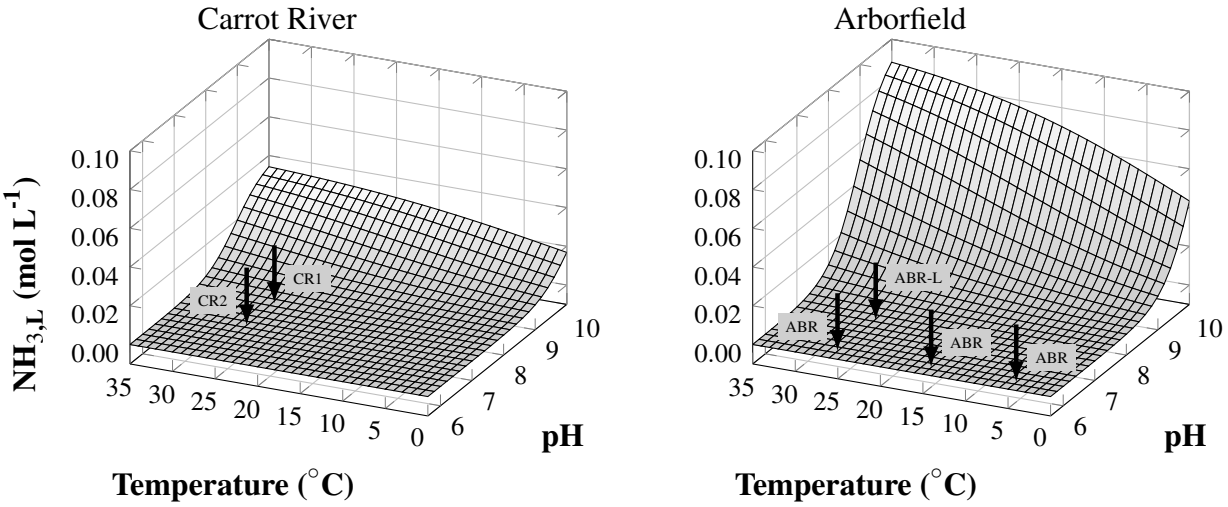


Fig. 5.8. Impact of pH and temperature on the predicted aqueous NH_3 concentration at the soil air-interface of Carrot River (CR) and Arborfield (ABR) soil used in the lab, according with Sherlock and Goh (1984) and Sommer et al. (2004) (Eq. 2.3) under the assumption that water content is homogeneously distributed within the soil and that 18.64 mg N of fertilizer N are completely dissolved and hydrolyzed within the first 1 cm of the soil. The concentration of ammoniacal N within the first 1 cm of the soil after application of N fertilizer was assumed to be 0.037 mol L^{-1} for Carrot River and 0.096 mol L^{-1} for Arborfield soil.

This was in agreement with the observed volatilization losses in Carrot River and Arborfield soils in the current study. When these two soils were compared under almost similar pH conditions, cumulative NH_3 losses from untreated urea in the ABR-L soil (pH 7.1) exceeded those in the CR2 soil (pH 6.9) by two-fold (See Table 5.5). Additionally, volatilization losses from the Arborfield soil at a gravimetric soil water content (GSWC) of 100% FC (0.31 g H_2O g $soil^{-1}$) were only half of those at 50% FC (Table 5.6), emphasizing the effect of increased soil moisture content on diluting TAN, therefore reducing NH_3 losses.

The Arborfield soil showed 12- to 17-times greater urease activity compared to the Carrot River soil (Fig. 5.7), which helps explain why NH_3 losses were higher from the Arborfield soil.

High urea hydrolysis rates can rapidly increase the TAN concentration at the soil surface, causing conditions that favor volatilization losses. This effect has been observed by Rochette et al. (2009a), who suggested that increased amounts of crop residues at the surface of no-till soils were important contributors to high urease activities. This was also true in the current study, where urease activity was higher in the soil with visibly higher plant residue content (i.e., the Arborfield soil).

Findings from the current study suggest that the effect of soil pH on NH_3 losses is likely only a secondary factor depending on urease activity when urea is used as a fertilizer, because even at a high soil pH, a low urea hydrolysis rate will limit the amount of ammoniacal N available for volatilization.

Fertilizer products containing urease inhibitors (i.e., UI-1, UI-2, and DI) showed clear trends in NH_3 volatilization patterns in the Carrot River soils. These stabilized fertilizers numerically reduced NH_3 losses relative to untreated urea, although only the DI (which included both a urease and nitrification inhibitor) reduced losses significantly in both Carrot River soils. This indicates that NBTPT-based urease inhibitors in combination with DCD-based nitrification inhibitors were generally efficient in reducing NH_3 losses. Losses associated with the NI, on the other hand, were elevated compared to urea. Similar results have been observed by Zaman et al. (2009), Soares et al. (2012), and Zaman et al. (2013), who found that application of the nitrification inhibitor DCD increased losses due to NH_3 volatilization relative to urea or urine without DCD. Indeed, the inhibition of nitrification can prolong the presence of TAN in the soil solution, thus increasing the likelihood of N losses due to volatilization. In those studies, however, it is likely that the high concentrations of fertilizer N (300 to 600 kg N ha^{-1}) were favoring NH_3 losses when the nitrification inhibitor was applied. Because nitrification inhibitors only indirectly affect NH_3 volatilization losses, they may have little impact on NH_3 volatilization when nitrification rates are low. This was underscored by Ni et al. (2014), who found that nitrification inhibitors showed no effect on NH_3 losses from surface applied urea. In the current study, the effectiveness of the stabilized fertilizers at reducing NH_3 losses from the Carrot River soils was poor (Fig. 5.7). This was likely due to the low urease activity of this soil, combined with the comparatively low N application rates.

Long-term incubation of the unfertilized (C) Carrot River soils had little effect on the residual soil N (NH_4^+ and NO_3^-), indicating that mineralization of soil organic N did not contribute significantly to soil N loads under the conditions of the experiment. All fertilizer products added similar amounts of residual N to the soils, with no difference between the stabilized fertilizers and untreated urea alone. Indeed, with low overall volatilization losses from the Carrot River soils, it is not surprising that all fertilizer products yielded similar residual N contents. Indeed, the majority of residual N from all fertilizers was available as NO_3^-, and stabilized fertilizer products containing nitrification inhibitors (i.e. NI and DI) showed no reduction in NO_3^- content compared to urea.

This indicates that most of the NH_4^+ released from fertilizers had been further transformed to NO_3^-, regardless of the presence of the nitrification inhibitors.

The DI and UI-2 products reduced NH_3 emissions in the non-limed Arborfield soil by up to 66% when compared to untreated urea. This effect was much stronger in the non-limed Arborfield soil than in Carrot River soils, indicating that the urease inhibitors were more effective at reducing NH_3 losses from soils with generally high loss potentials.

Interestingly, at a pH of 7.1 (ABR-L), both UI-1 and UI-2 showed no significant difference in cumulative NH_3 volatilization losses compared to urea, though the UI-2 delayed NH_3 volatilization losses by 24 h, compared to urea. The only stabilized fertilizer that significantly reduced NH_3 volatilization losses in the ABR-L soil was the DI. It is possible that the difference in NH_3 losses between UI-1 and DI resulted from differences in application rate and type of the inhibitor NBTPT (Table 5.2). While information about the concentration of NBTPT in the DI is not available, it is likely that it exceeded the concentration coated on the urea in the UI-1 product. This was reflected by consistently lower urea hydrolysis rates from DI in all soils, although they were only significantly reduced from UI-1 in native Arborfield soil (Fig. 5.7). Conversely, DI consistently resulted in the lowest NH_3 volatilization losses within every soil and under every soil conditions (i.e., soil moisture content and temperature), except for CR2 soil, where it shared the lowest emissions with UI-1.

The incorporation of NBTPT into the granules of the DI during production of the fertilizer is likely another reason why NH_3 losses from the DI were low in all experiments. Incorporation results in a more homogeneous distribution of the inhibitor within the granule. In the current study, granule size differed between products, but to ensure consistency, two granules totaling 18.64 mg N were added to each soil chamber. Under these conditions, variations in coating density between UI-1 granules could have affected the amount of inhibitor that was introduced into the volatilization chambers, potentially decreasing its effect on NH_3 losses when granules with less dense coating were selected. On the other hand, the incorporation of the urease inhibitor with the urea in the DI is likely to have resulted in more even inhibitor concentrations throughout all granules, therefore reducing the impact of variations in inhibitor concentrations on NH_3 losses.

The effect of uneven coating of UI-1 is likely to decrease as the amount of product used for the experiment is increased. This was supported by results from the urea hydrolysis experiment (Fig. 5.7). Similar to the DI, the product UI-1 reduced urea hydrolysis rates significantly compared to urea, likely because large number of granules (250 to 350 granules per litre) were used to prepare the fertilizer solution, reducing the impact of variation in coating density of individual granules on the total available inhibitor concentration in solution. The small size of the soil chambers and the limitation in available granule sizes, however, made it impossible to increase application rate of granules within the volatilization chambers while keeping the N application rate similar to rates

used in the field. This may explain, why variations in the reduction in NH_3 volatilization losses by UI-1 were observed, although UI-1 showed a similar inhibitory effect as DI and UI-2.

Total residual N added by fertilizer products was numerically higher in the native (ABR) than in limed Arborfield soil (ABR-L). Total NO_3^- concentrations did not differ significantly among fertilizer products in either trial, but were more variable in the first trial of the native ABR soil (i.e., 26°C, 75% FC). Stabilized fertilizers containing nitrification inhibitors (i.e., NI and DI) consistently increased the residual NH_4^+ content of the soil, compared to all other fertilizers, and this effect was stronger at low pH. This was expected, as nitrification inhibitors block the transformation of ammoniacal N to NO_3^-, thereby preserving a larger fraction of N in the ammoniacal N pool. This effect was significant for the DI, compared to fertilizers without nitrification inhibitors (i.e., urea, UI-1, and UI-2) in both the native and limed Arborfield soils. The NI showed a similar trend in preserving the ammoniacal N pool, but NH_4^+ concentrations were significantly higher than those associated with the untreated urea in limed Arborfield soil only. It is likely that residual NH_4^+ concentrations in the soil were lower with the NI than the DI, because the DI also contains a urease inhibitor. Therefore, the DI limited urea hydrolysis and reduced subsequent NH_3 volatilization losses, while N added with the NI was not protected against urea hydrolysis. This resulted in significantly increased NH_3 volatilization from the NI compared to the DI, and is likely the reason for the reduced NH_4^+ content in soil treated with the NI.

The stabilized UI-2, and DI fertilizers, both containing a urease inhibitor, generally reduced NH_3 volatilization losses under different moisture conditions. This effect was apparent at 50% FC (Fig. 5.5a) and intensified at 75% FC (Fig. 5.5b), where UI-2, and DI reduced volatilization losses relative to urea. The efficiency of stabilized fertilizers in reducing NH_3 losses, however, declined at 100% FC (Fig. 5.5c), resulting in no significant differences among any fertilizer products and urea. This is not surprising, as higher soil moisture contents dilute the concentration of TAN at the soil surface, resulting in a lower volatilization loss potential of the soil. Moreover, nitrification rates were likely enhanced at higher soil moisture contents, reducing the NH_3 available for volatilization. This is in agreement with Di et al. (2014), who demonstrated that nitrification rates from urea increase with increasing soil moisture content from 60% FC to 100% and 130% FC. In the current study, soil moisture contents of 50% FC reduced the efficiency of stabilized fertilizers relative to 75% FC. This was likely caused by lower urea hydrolysis rates at low soil moisture conditions. These results suggest that the efficacy of stabilized fertilizers in reducing NH_3 losses is enhanced under soil conditions that generally exhibit strong NH_3 loss potentials.

Residual N contents of the soils demonstrated the effect of nitrification inhibitors in preserving ammoniacal N within the soils. The soil ammoniacal N pool from application of urea decreased consistently with rising soil moisture content. This is likely a result of increased nitrification rates under increased soil moisture conditions. The fertilizer products containing nitrification inhibitors

(i.e., NI and DI) successfully prevented the conversion of TAN to NO_3^-, as reflected by significantly increased NH_4^+ contents from these products. Furthermore, the effect of preserving TAN was strongest at 100% FC, resulting in four times higher NH_4^+ contents where NI and DI were applied, compared to urea. Similar results were reported by Di et al. (2014), who found that the nitrification inhibitor DCD was most successful in preserving ammoniacal N at high soil moisture contents (i.e., 100% and 130% FC).

Stabilized fertilizers containing urease inhibitors (i.e., UI-2 and DI) reduced NH_3 losses consistently at all tested temperatures. Volatilization losses from DI were almost completely absent at 5°C, whereas urea showed similar cumulative losses at 5°C as at 15°C. This indicated that temperature had no effect on the efficiency of urease inhibitors within this temperature range. Volatilization losses from DI were lowest among all stabilized fertilizers containing urease inhibitors, possibly due to homogeneous distribution and a high concentration of the inhibitor within the granules. Losses from UI-1 were not different from urea, possibly a result of a relatively lower concentration of NBTPT within the coating (i.e., 1.5 g kg^{-1} urea).

Residual N contents in the soil demonstrated the effect of nitrification inhibitors on the TAN pool. At 5°C, temperatures were likely too low to permit significant nitrification, because the optimum for nitrification lies between 25°C and 35°C (Saad and Conrad, 1993). This was reflected by similar NH_4^+ contents of the soil among all fertilizer products, regardless of the applied inhibitors. As temperatures were increased to 15°C and 26°C, the proportion of NH_4^+ in the residual N pool decreased more strongly in fertilizers that did not contain nitrification inhibitors, whereas the products NI and DI preserved a numerically larger fraction of ammoniacal N in the soil.

5.7 Conclusions

The series of bench-scale volatilization experiments demonstrated that stabilized fertilizer products containing a double inhibitor (DI) were able to reduce NH_3 losses compared to untreated urea (U) across a range of soil conditions, whereas fertilizers containing a nitrification inhibitor only (i.e., NI) generally showed no reduction NH_3 losses. The fact that UI-1, UI-2 and DI reduced NH_3 losses but did not result in higher residual N contents showed that other loss processes such as denitrification or microbial immobilization were likely affecting the residual N pool. As a result, products containing a nitrification inhibitor (i.e., NI and DI) increased the proportion of residual NH_4^+ in the soil.

Differences in soil pH only affected NH_3 losses in one soil and were likely only a secondary factor governing emission losses. Manipulations of soil moisture content affected both the magnitude of NH_3 losses and the composition of residual N (i.e., NH_4^+/NO_3^-), likely as a result of insufficient urea hydrolysis at low moisture contents (e.g., 50% FC) and enhanced dilution of urea

at high moisture contents (e.g., 100% FC). Consequently, low moisture contents reduced total residual N and preserved relatively more NH_4^+, whereas high moisture contents increased total residual N and resulted in the near complete transformation of ammoniacal N to NO_3^-.

Low soil temperature (i.e., 5°C and 15°C) delayed the onset of NH_3 volatilization relative to 26°C, likely as a result of slowed hydrolysis of urea, as reflected by increased proportions of residual NH_4^+ at low temperatures. Stabilized fertilizers containing a combination of urease and nitrification inhibitors (i.e., DI) were most successful in mitigating NH_3 losses.

Urease activity varied strongly between soils and was likely one of the main drivers for NH_3 volatilization losses. When urea hydrolysis rates of stabilized fertilizers were assessed within both soils, those products containing urease inhibitors (i.e., UI-1, UI-2, and DI) reduced urea hydrolysis rates significantly when the native soil urease activity was high, but were not different from untreated urea when the soil urease activity was low. This indicated that the efficacy of stabilized fertilizers containing urease inhibitors may be greater when soil urease activity is high. Urease activity of the soil should therefore be considered to be included in standard soil tests when uncertainties exist about the usefulness of stabilized urea fertilizers.

6 SYNTHESIS AND CONCLUSIONS

The gaseous loss of N from urea-based and ammoniacal fertilizers is a well known phenomenon (Kissel et al., 1977; Sommer et al., 2004). Gaseous N losses as NH_3 and N_2O from applied fertilizers can significantly reduce fertilizer-use efficiency and pose a threat to the environment through promotion of the greenhouse gas effect and the eutrophication and acidification of ecosystems (Sommer et al., 2004). The fertilizer industry has responded by developing urease- and nitrification inhibitors that can reduce gaseous N losses when applied alone or together with urea-N (Trenkel, 2010). Research has focused primarily on the efficacy of urease- and nitrification inhibitors in reducing NH_3 and N_2O emissions, and nitrate leaching in a large variety of soils under different climatic and management conditions. This research has shown that soils vary in their potential for gaseous N losses from applied urea due to differences in biotic and abiotic factors (Sommer et al., 2004), and while the impact of these factors on gaseous N emissions is well understood, less is known about how these factors affect the efficacy of urease- and nitrification inhibitors in reducing emission losses. The research presented in this dissertation addresses some of the research gaps related to the impact of biotic and abiotic factors on gaseous N losses from soils in the Boreal Transition Zone of Saskatchewan.

The goals of this study were to (i) determine the potential of urease and nitrification inhibitors to reduce losses of NH_3 and N_2O from agricultural soils managed for the production of seed from perennial forage grasses in Saskatchewan and (ii) assess the impact of environmental factors, such as soil pH, moisture content, and temperature, on the performance of urease and nitrification inhibitors in reducing NH_3 losses under controlled conditions. Furthermore, a novel and cost-efficient chamber-based system for measuring NH_3 losses in the field was developed for use in this study.

6.1 Summary of Findings

Determining the potential for urease- and nitrification inhibitors to reduce gaseous NH_3 losses under field conditions in remote sites, such as in forage seed production sites in the Boreal Transition Zone of Saskatchewan, is challenging, because it requires a high number of replicated mea-

surements within a relatively small area. Moreover, chamber- or wind tunnel systems suitable for this type of measurement usually require in-field current, and are costly and laborious (Sommer et al., 2004). In the first study (Chapter 3), a novel cost-effective system for measuring NH_3 emissions in remote sites was developed and validated under field conditions using three rates of surface-applied urea (i.e., 0, 46, and 92 kg N ha^{-1}). Although cumulative losses from the application of 92 kg urea-N ha^{-1} during a 10-d measurement period only totaled 1.12% of applied N, the system was able to detect significant differences between all application rates. The recovery rate of gaseous NH_3 within the chamber system also was determined in the lab under controlled conditions, and demonstrated that a relatively short sampling period (i.e., 90 min) was sufficient for detecting differences in NH_3emissions between treatments in the field.

One of the objectives of this study was to determine how effective urease and nitrification inhibitors were at reducing gaseous NH_3 and N_2O losses from agricultural soils managed for forage seed production in the Boreal Transition Zone of Saskatchewan (Chapter 4). This research demonstrated that the NH_3 loss potential from untreated urea was higher after a spring application than after a fall application. Moreover, the NH_3 loss potential of the CR1 site—with a higher pH and soil moisture content than the CR2 site—was greater (141.2 g N ha^{-1} d^{-1}) than that at the CR2 site (41.5 g N ha^{-1} d^{-1}). Following a fall application in 2012, the loss potential was negligible immediately after application and during the subsequent spring snowmelt. This was likely a result of low temperatures during the fall and infiltration of any remaining ammoniacal N into the soil during snowmelt. In the fall of 2013, temperatures during the sampling period were higher as was the NH_3 loss potential, compared to the previous fall. Stabilized fertilizers containing urease inhibitors (i.e., UI-1 and DI) reduced gaseous NH_3 losses significantly after application in the spring and in the fall of 2013 when the loss potential of the soil was higher (i.e., at the high-pH site), but were no different than untreated urea when the loss potential of the soil was low (i.e., at the low-pH site and in the fall of 2012). Among all fertilizer products, those containing a double inhibitor (DI) consistently resulted in the lowest NH_3 emissions.

Nitrous oxide emissions from fall-applied fertilizers were highest immediately after snowmelt—reflecting the formation of ideal conditions for denitrification—whereas emissions during the fall were negligible. After snowmelt, the products containing a nitrification inhibitor consistently reduced N_2O emissions from all sites, compared to emissions from untreated urea and the fertilizer product containing only a urease inhibitor (i.e., UI-1). After a spring fertilizer application, however, N_2O emissions from stabilized fertilizers differed strongly among sites. At the Carrot River sites (CR1 and CR2), emissions of N_2O from untreated urea showed a pattern similar to that observed for NH_3 emissions; i.e., emissions were greater at the site with a higher moisture content and pH (CR1). At CR1, all the stabilized fertilizer products were able to reduce N_2O emissions relative to untreated urea, with emissions from products containing a nitrification inhibitor

(i.e., DI and NI) being lowest. At the ABR and CHL sites N_2O emissions from untreated urea were relatively low (7.6 and 4.8 g N ha^{-1} d^{-1}, respectively) and the products containing a urease inhibitor (UI-1 and DI) increased N_2O losses relative to urea, ranging from 11.4 to 22.8 g N ha^{-1} d^{-1}.

This study showed that application timing (i.e., fall *vs.* spring) had a strong impact on type and magnitude of gaseous N emissions. The majority of gaseous NH_3 emissions can be mitigated when fertilizers are applied in the fall, but this increases the potential for gaseous N_2O losses in the following spring under conditions common in the Boreal Transition Zone of Saskatchewan. Applying stabilized fertilizers that contain double inhibitors in the fall has the potential to reduce both NH_3 emissions during the fall and N_2O emissions during the following spring snowmelt. On the other hand, application of fertilizers in the spring greatly increases the potential for NH_3 emissions due to higher temperatures and when precipitation is lacking. Using fertilizer products that contain urease inhibitors can strongly reduce these losses when the loss potential of the soil is high, but are likely to show no significant difference to untreated urea when the NH_3 loss potential of the soil is low.

As demonstrated in chapter 4, differences in soil properties, such as pH, water content, and temperature at the time of application (i.e., fall *vs.* spring) can strongly affect type and magnitude of gaseous N losses, and thus the performance of stabilized fertilizers in reducing these losses. However, because field sites often differ in several of these properties at the same time, it is difficult to assess the contribution of each of these factors on the performance of stabilized fertilizers.

One objective of this research was to determine the impact of soil pH, moisture content, and temperature on the performance of stabilized fertilizers in reducing NH_3 volatilization losses under controlled soil environmental conditions (Chapter 5). In doing so, NH_3 losses were more affected by the soil (i.e., Carrot River *vs.* Arborfield) than by changes in soil pH within each soil. Interestingly, the soil with the lowest pH (i.e., Arborfield) showed highest NH_3 losses. This was surprising, as NH_3 losses are known to increase with increasing pH (Sommer et al., 2004). However, the Arborfield soil also showed significantly ($P < 0.001$) higher urea hydrolysis rates than the Carrot River soil, which might have led to a more rapid increase in NH_4^+/NH_3 concentration at the soil surface, thus favoring NH_3 losses.

Additionally, at the set water content of 75% FC, the Arborfield soil contained less water than the Carrot River soils (i.e., 0.23 *vs.* 0.41 and 0.37 g H_2O g $soil^{-1}$, respectively), thus resulting in greater concentrations of ammoniacal N after application of 18.64 mg N. Because NH_3 volatilization depends on the concentration of ammoniacal N at the soil surface, this might have contributed to the increased NH_3 volatilization from Arborfield soil. These findings indicate that in soils rich in organic matter, such as chernozems within the Boreal Transition Zone of Saskatchewan, soil urease activity and moisture content may be more important than pH in affecting NH_3 losses from surface-applied urea fertilizers.

Differences in the pH of the Carrot River soil did not affect the magnitude of NH_3 losses from any of the fertilizer products tested under lab conditions. Those products containing either a urease or a double inhibitor were able to reduce NH_3 losses compared to urea. Altering the pH of Arborfield soil, on the other hand, affected both the magnitude of NH_3 emissions from the fertilizer products as well as the performance of stabilized fertilizers. In the native (i.e., non-limed) Arborfield soil, products containing either a urease or double inhibitor reduced NH_3 losses significantly, whereas under limed conditions, only the fertilizer product containing a double inhibitor decreased NH_3 losses significantly

The magnitude of NH_3 losses as well as the performance of stabilized fertilizers were affected by the soil moisture content. Ammonia losses from urea increased from 10.7% at 50% FC to 22.2% at 75% FC, but were reduced to 5.8% at 100% FC. Fertilizer products containing a urease inhibitor were able to reduce NH_3 losses significantly under conditions where NH_3 losses were generally higher (i.e., at 50 and 75% FC), but not at 100% FC, where NH_3 losses were low. On the other hand, fertilizer products containing only a nitrification inhibitor did not affect NH_3 losses compared to urea.

Differences in soil and air temperature affected the magnitude and temporal patterns of NH_3 emissions from untreated urea and the stabilized fertilizer products. The onset of NH_3 losses from untreated urea was delayed by up to 120 h at 5°C, and this delay was shortened to 48 h when temperature was increased to 15°C. At 26°C, the lag period was further reduced to 12 h and the magnitude of NH_3 losses from urea was increased to 22.2% of the applied N. Stabilized fertilizers containing a double inhibitor reduced NH_3 losses at all temperatures, whereas the effect from products containing a urease inhibitor only (i.e., UI-1 and UI-2) was apparent only at 26°C. Whereas fertilizer products containing nitrification inhibitors significantly increased soil residual NH_4^+ at 15 and 26°C, this effect was not observed at 5°C, demonstrating that low temperatures reduce the potential for nitrification.

The results from Chapter 5 demonstrated that the soils differed in their potential for NH_3 volatilization, and that stabilized fertilizers are most effective in reducing these losses under conditions where the NH_3 loss potential of the soil is high. To further determine the efficacy of stabilized fertilizers in reducing urea hydrolysis in the soils used for the bench-scale volatilization experiments, the urea hydrolysis rates in presence of the stabilized fertilizer products were assessed according to Kandeler and Gerber (1988). In doing so, the native Arborfield soil showed significantly ($P < 0.001$) increased urea hydrolysis rates (79 μg NH_4^+-N g soil^{-1} 2 h^{-1}) compared to the Carrot River soils (5.5 to 6.6 μg NH_4^+-N g soil^{-1} 2 h^{-1}). Stabilized fertilizers containing urease inhibitors (i.e., UI-1, UI-2, and DI) yielded urea hydrolysis rates equivalent to that for the untreated urea in the Carrot River soils, but significantly reduced urea hydrolysis rates in the Arborfield soil, and this effect was stronger when the soil pH was higher.

6.2 Overall performance of the stabilized fertilizers

The overall performance of the stabilized fertilizers in reducing gaseous NH_3 and N_2O losses across a range of environmental conditions (e.g., fall *vs.* spring) was compared using non-metric multidimensional scaling (NMDS). Non-metric multidimensional scaling is an ordination method that displays similarities or dissimilarities between samples as distances within the ordination space. Although originally intended to answer psychological questions, the use of NMDS soon expanded into the field of ecology and other disciplines, due to the large flexibility of NMDS (Clarke and Warwick, 1994). The basic algorithm of NMDS starts by calculating a similarity matrix between the profiles of properties of samples, and transforming these similarities into ranks. Using a random starting configuration, the program then tries to "map" samples in the ordination space according to the rank similarity matrix. The result is a plot (often two-dimensional) in which distances between samples in the ordination space correspond to their rank in similarity. For example, if the rank similarity between sample *A* and sample *B* is higher than between sample *A* and sample *C*, then sample *A* will be placed closer to sample *B* than to sample *C*, etc.

In this study, the cumulative emissions of a treatment across all environmental conditions (i.e., field measurement periods and field sites, or bench scale experiments) were summarized as its "emission profile" (Appendix A.3). In doing so, it was possible to compare the general performance of stabilized fertilizer products across environmental conditions. Furthermore, by including the unfertilized control in the ordination, it was possible to determine which stabilized fertilizer was closest to the unfertilized control in its "emission profile".

When the overall performance of the stabilized fertilizers for reducing NH_3 volatilization losses was assessed in the field, there was a clear trend in gaseous losses between fertilizer products (Fig. 6.1). In the ordination, products containing a double inhibitor were most similar to the unfertilized control, followed by products containing a urease or a nitrification inhibitor only. Untreated urea, on the other hand, was most different from the unfertilized control. This was expected, as emissions from untreated urea were highest most of the time, while background levels from the unfertilized control were generally negligible. As a result, the difference between the unfertilized control and the urea treatment were greatest. Products containing a double inhibitor, on the other hand, yielded the smallest emissions during all experiments, and were thus most similar to the unfertilized control. The product containing a nitrification inhibitor only reduced NH_3 losses relative to untreated urea in a few trials, and was thus more similar to untreated urea than to the unfertilized control.

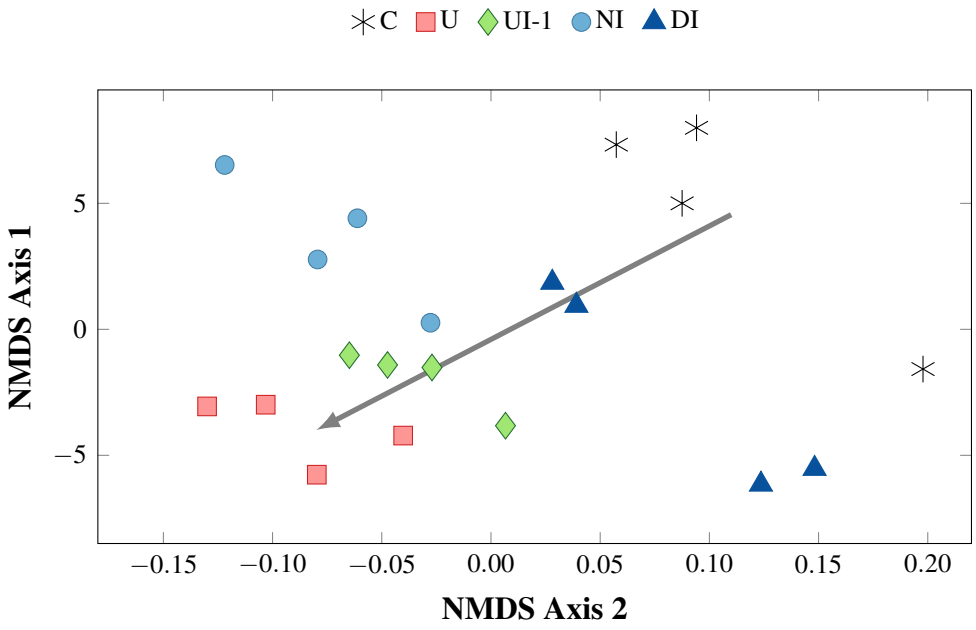


Fig. 6.1. Non-metric multidimensional scaling of the cumulative NH_3 emission profiles of the four replicates of different stabilized fertilizer treatments under field conditions (Stress = 8.65). C = unfertilized control, U= untreated urea, UI-1= urease inhibitor (NBTPT), NI = nitrification inhibitor (DCD + TZ), DI = double inhibitor (NBTPT + DCD). The arrow indicates the direction of the strongest increase in cumulative NH_3 emissions (e.g., NH_3 emissions were greatest from U and lowest from DI and C).

The performance of stabilized fertilizer products during bench-scale volatilization experiments showed a similar trend to what was observed in the field, although all fertilizer products clustered further away from the unfertilized control (Fig. 6.2). This was not surprising, given the ratio of NH_3 emissions to those from the unfertilized control (i.e., background emissions) was greater during the bench-scale volatilization experiments than during the field experiments, likely reflecting the more complete recovery of NH_3 under lab conditions. As a result, the distance in the ordination space between all fertilizer products and the unfertilized control was larger than in the ordination from field measurements, where emissions were not measured continuously. Among all treatments, the product containing a double inhibitor (i.e., DI) was closest to the unfertilized control, followed by the products containing a urease inhibitor only (i.e., UI-2 and UI-1). Untreated urea and the product containing a nitrification inhibitor (i.e., U and NI) were most different from the unfertilized control.

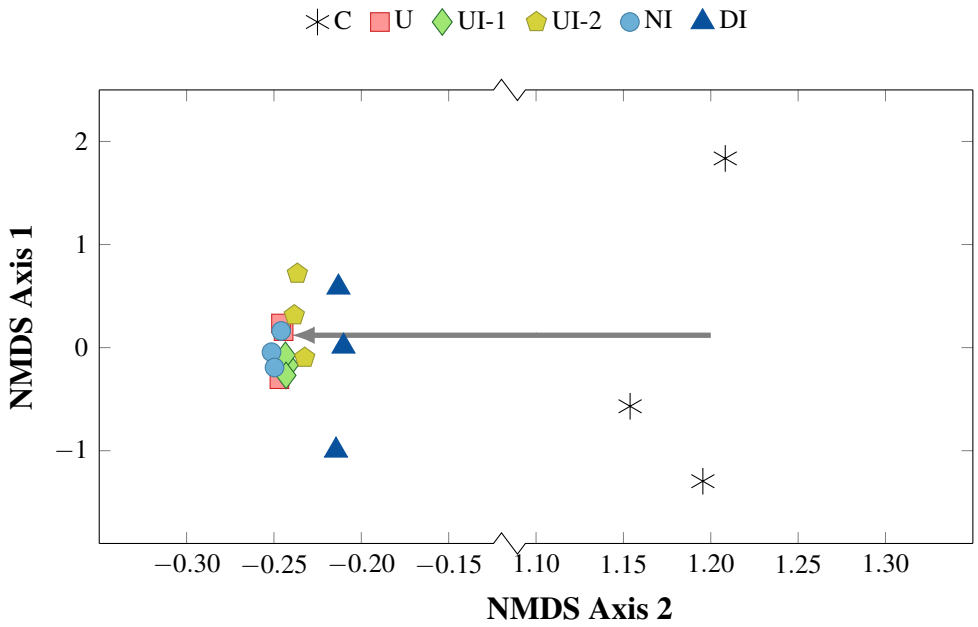


Fig. 6.2. Non-metric multidimensional scaling of the cumulative NH_3 emission profiles of the three replicates of different stabilized fertilizer treatments during bench-scale volatilization experiments (Stress = 0.16). C = unfertilized control, U= untreated urea, UI-1= urease inhibitor (NBTPT), NI = nitrification inhibitor (DCD + TZ), DI = double inhibitor (NBTPT + DCD). The arrow indicates the direction of the strongest increase in cumulative NH_3 emissions (e.g., NH_3 emissions were greatest from U and NI and lowest from DI and C).

The impact of stabilized fertilizers on N_2O emission reductions in the field was affected by more variability than emissions of NH_3. Nevertheless, stabilized fertilizer products containing nitrification inhibitors (i.e., DI and NI) were more similar to the unfertilized control than the fertilizer products containig no nitrification inhibitors (Fig. 6.3).

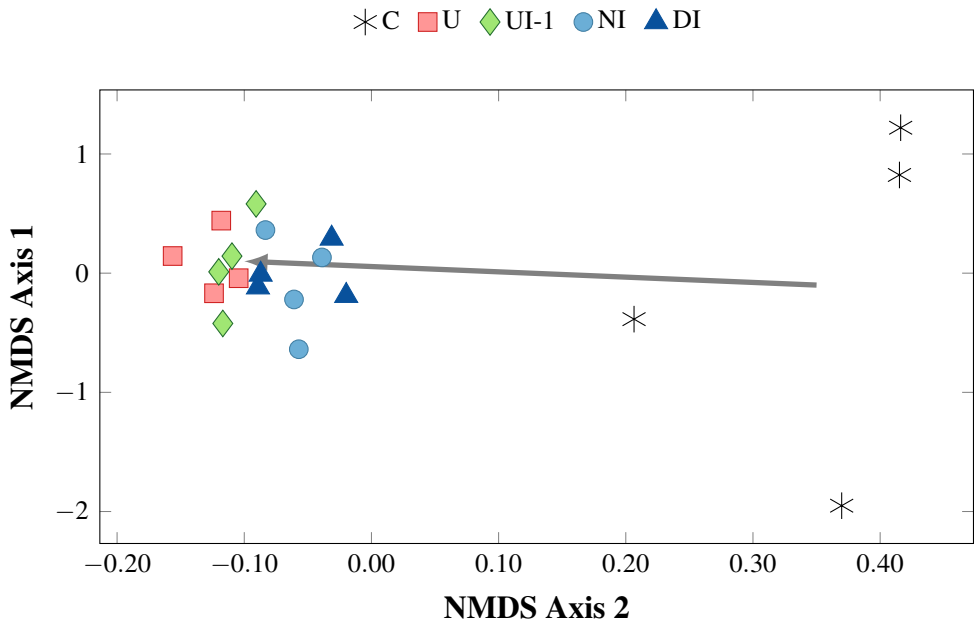


Fig. 6.3. Non-metric multidimensional scaling of the cumulative N_2O emission profiles of the four replicates of different stabilized fertilizer treatments under field conditions (Stress = 4.96). C = unfertilized control, U= untreated urea, UI-1= urease inhibitor (NBTPT), NI = nitrification inhibitor (DCD + TZ), DI = double inhibitor (NBTPT + DCD). The arrow indicates the direction of the strongest increase in cumulative N_2O emissions (e.g., N_2O emissions were greatest from U and lowest from C).

This research indicated that the use of a double inhibitor is most promising under the environmental conditions common to the Boreal Transition Zone of Saskatchewan. This effect was stronger for ammonia losses than for N_2O losses. Furthermore, the bench-scale NH_3 volatilization experiments revealed that the Arborfield site was more prone to NH_3 losses due to its reduced water-holding capacity and increased soil urease activity.

6.2.1 Future Research

In this study, soil environmental conditions of field sites in close proximity resulted in different N loss potentials. The novel approach of using short-term urease activity assays to test the inhibitory effect of stabilized fertilizers in a given soil proved successful in helping predict potential N losses. If a similar approach was used to assess the products' effect on nitrification rates in addition to urease activity rates, this would provide a tool for (a) assessing the potential losses of N through emissions of either NH_3 or N_2O, and (b) estimating how well each stabilized fertilizer product would be suited in reducing the respective N losses.

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APPENDICES

APPENDIX A CALCULATIONS

A.1 Example calculation of the model by Sherlock and Goh (1984)

The model by Sherlock and Goh (1984) (Eq. 2.3) was calculated for Carrot River and Arborfield soil (Fig. 5.8), assuming urea hydrolysis was complete and the soil water content evenly distributed within the soil. The height of the sieved (< 2mm) CR1 and CR2 soils in the reaction chambers was 3.5 cm, the height of the sieved ABR soil was 5 cm. At 75% FC, the soil moisture content was 0.41, 0.37, and 0.23 g H_2O g^{-1} soil for CR1, CR2, and ABR soil, respectively. Using CR1 soil as an example, the surface 1-cm of the soil would contain 35.143 g H_2O (0.035143 L):

$$0.41 \text{ g } H_2O \text{ g}^{-1} \text{ soil} \times 300 \text{ g soil} : 3.5 \text{ cm} = 35.143 \text{ g } H_2O = 0.035143 \text{ L}$$

The addition of 18.64 mg N (0.01864 g N) would then increase the N concentration in the surface 1-cm of the soil to 0.038 mol L^{-1}:

$$0.01864 \text{ g N} : 14.0067 \text{ g mol}^{-1} : 0.035143 \text{ L} = 0.038 \text{ mol L}^{-1}$$

With the TAN concentration of 0.038 mol N L^{-1} at a temperature of 35°C (308.15 K) and a pH of 10, the model would predict a concentration of 0.035 mol L^{-1} NH_3–N (See Fig. 5.8):

$$\frac{0.038 \text{ mol L}^{-1}}{1 + 10^{(0.09018 \, + \, 2729.92 \, / \, 308.15 \, - \, 10)}} = 0.035 \text{ mol NH}_3\text{-N L}^{-1}$$

A.2 ANOVA tables and soil residual N levels

Table A.1. ANOVA table for the factorial design with average daily N_2O emissions as the dependent variable and fertilizer product, test site, and time of application (i.e., fall or spring) as the main factors.

Factor	Degrees of freedom	Sum of squares	Mean squares	*F*-value	*P*-value
Fertilizer	4	122.2	30.5	42.6	**< 0.001**
Site	3	7.4	2.5	3.4	**0.02**
Time	1	2.15	2.15	3.0	0.09
Fertilizer × Site	12	20.6	1.7	2.4	**0.008**
Fertilizer × Time	4	6.7	1.7	2.3	0.06
Site × Time	3	23.7	7.9	11.0	**< 0.001**
Fertilizer × Site × Time	12	14.9	1.2	1.7	0.06
Residuals	120	86.1	0.78		

Table A.2. ANOVA table for average daily NH_3 losses from spring applied fertilizers (2013) in a factorial design with fertilizer product and test site as the main factors.

Factor	Degrees of freedom	Sum of squares	Mean squares	*F*-value	*P*-value
Fertilizer	4	20.4	5.11	29.1	**< 0.001**
Site	1	5.3	5.3	30.3	**< 0.001**
Fertilizer × Site	4	3.6	0.9	5.1	**0.003**
Residuals	30	5.28	0.2		

Table A.3. ANOVA table for average daily NH_3 losses from fall applied fertilizers (2013) in a factorial design with fertilizer product and test site as the main factors.

Factor	Degrees of freedom	Sum of squares	Mean squares	*F*-value	*P*-value
Fertilizer	4	16.5	4.1	17.2	**< 0.001**
Site	1	3.2	3.2	13.3	**< 0.001**
Fertilizer × Site	4	5.5	1.4	5.8	**0.001**
Residuals	30	7.2	0.2		

Table A.4. ANOVA table for the cumulative NH_3 losses from stabilized fertilizer products applied to Carrot River soil in a factorial design with fertilizer product and soil pH as the main factors.

Factor	Degrees of freedom	Sum of squares	Mean squares	*F*-value	*P*-value
Fertilizer	4	3.06	0.77	20.3	**< 0.001**
pH	1	0.004	0.004	0.1	0.76
Fertilizer × pH	4	0.18	0.04	1.17	0.35
Residuals	20	0.75	0.04		

Table A.5. ANOVA table for the cumulative NH_3 losses from stabilized fertilizer products applied to Arborfield soil in a factorial design with fertilizer product and soil pH as the main factors.

Factor	Degrees of freedom	Sum of squares	Mean squares	*F*-value	*P*-value
Fertilizer	4	25.6	6.4	10.8	**< 0.001**
pH	1	0.62	0.62	1.04	0.32
Fertilizer × pH	4	7.4	1.8	3.1	**0.038**
Residuals	20	11.8	0.59		

Table A.6. ANOVA table for the cumulative NH_3 losses from stabilized fertilizer products applied to Arborfield soil in a factorial design with fertilizer product and soil moisture content as the main factors.

Factor	Degrees of freedom	Sum of squares	Mean squares	*F*-value	*P*-value
Fertilizer	4	8.16	2.04	12.85	**< 0.001**
Moisture	1	3.30	3.30	20.8	**< 0.001**
Fertilizer × Moisture	4	0.45	0.11	0.71	0.59
Residuals	35	5.55	0.16		

Table A.7. ANOVA table for the cumulative NH_3 losses from stabilized fertilizer products applied to Arborfield soil in a factorial design with fertilizer product and soil temperature as the main factors.

Factor	Degrees of freedom	Sum of squares	Mean squares	*F*-value	*P*-value
Fertilizer	4	3.40	0.85	16.4	**< 0.001**
Temperature	1	1.95	1.95	37.5	**< 0.001**
Fertilizer × Temperature	4	0.25	0.06	1.21	0.32
Residuals	35	1.82	0.05		

Table A.8. Residual NH_4^+-N and NO_3^--N in excess to the unfertilized control from stabilized fertilizer products applied to Carrot River soil. Different lowercase letters indicate significant differences between means groups at $P < 0.05$.

		Fertilizer product‡					
Soil†	**pH**	**U**	**UI-1**	**UI-2**	**NI**	**DI**	**Mean**
		NO_3^--N (mg)					
CR2	6.87	11.33 ± 0.36	12.22 ± 3.04	11.43 ± 2.51	9.92 ± 2.49	11.52 ± 1.86	11.28
CR1	7.75	12.40 ± 1.47	11.94 ± 2.36	10.61 ± 1.27	9.81 ± 0.54	11.66 ± 0.96	11.28
Mean		11.87	12.07	11.02	9.87	11.59	
		NH_4^+-N (mg)					
CR2	6.87	-0.27 ± 0.06	-0.17 ± 0.16	-0.03 ± 0.32	0.21 ± 0.53	0.01 ± 0.42	-0.05
CR1	7.75	0.06 ± 0.81	-0.19 ± 0.15	-0.13 ± 0.54	-0.11 ± 0.51	-0.12 ± 0.33	-0.09
Mean		-0.11	-0.18	-0.08	0.05	-0.11	

† CR1/CR2= Carrot River soil

‡ U = untreated urea, UI-1 = urease inhibitor (NBTPT), UI-2 = urease inhibitor (2-NPT), NI = nitrification inhibitor (DCD + TZ), DI = double inhibitor (NBTPT + DCD).

Table A.9. ANOVA table for residual NO_3^--N in excess to the unfertilized control from stabilized fertilizer products applied to Carrot River soil in a factorial design with fertilizer product and soil pH as the main factors.

Factor	**Degrees of freedom**	**Sum of squares**	**Mean squares**	***F*-value**	***P*-value**
Fertilizer	4	18.81	4.70	1.31	0.30
pH	1	0.00	0.00	0.00	0.99
Fertilizer × pH	4	2.90	0.73	0.20	0.93
Residuals	20	71.92	3.60		

Table A.10. ANOVA table for residual NH_4^+-N in excess to the unfertilized control from stabilized fertilizer products applied to Carrot River soil in a factorial design with fertilizer product and soil pH as the main factors.

Factor	Degrees of freedom	Sum of squares	Mean squares	*F*-value	*P*-value
Fertilizer	4	0.13	0.03	0.18	0.95
pH	1	0.07	0.07	0.366	0.55
Fertilizer × pH	4	0.25	0.06	0.34	0.84
Residuals	20	3.62	0.18		

Table A.11. Residual NH_4^+-N and NO_3^--N in excess to the unfertilized control from stabilized fertilizer products applied to native and limed Arborfield soil. Different lowercase letters indicate significant differences between means groups at $P < 0.05$.

		Fertilizer product‡					
Soil†	**pH**	**U**	**UI-1**	**UI-2**	**NI**	**DI**	**Mean**
		NO_3^--N (mg)					
ABR	5.86	19.85 ± 10.57	20.32 ± 15.13	20.01 ± 9.68	5.28 ± 8.13	6.99 ± 9.91	14.61
ABR-L	7.07	10.52 ± 0.47	9.11 ± 1.35	10.84 ± 1.25	7.48 ± 2.61	8.53 ± 0.86	9.30
Mean		15.19	14.71	15.42	6.67	7.76	
		NH_4^+-N (mg)					
ABR	5.86	2.15 ± 1.24	1.57 ± 0.92	2.44 ± 2.01	4.23 ± 0.39	6.92 ± 1.42	3.46 a
ABR-L	7.07	-0.60 ± 0.38	0.11 ± 0.55	-0.40 ± 0.54	0.72 ± 0.58	1.82 ± 0.20	0.33 b
Mean		0.77 b	0.84 ab	1.02 ab	2.48 ab	4.37 a	

† ABR = Arborfield soil, ABR-L = limed Arborfield soil.

‡ U = untreated urea, UI-1 = urease inhibitor (NBTPT), UI-2 = urease inhibitor (2-NPT), NI = nitrification inhibitor (DCD + TZ), DI = double inhibitor (NBTPT + DCD).

Table A.12. ANOVA table for residual NO_3^--N in excess to the unfertilized control from stabilized fertilizer products applied to native and limed Arborfield soil in a factorial design with fertilizer product and soil pH as the main factors.

Factor	Degrees of freedom	Sum of squares	Mean squares	*F*-value	*P*-value
Fertilizer	4	453.3	113.32	1.89	0.15
pH	1	211.4	211.4	3.53	0.07
Fertilizer × pH	4	241	60.3	1.00	0.43
Residuals	20	1197.9	59.89		

Table A.13. ANOVA table for residual NH_4^+-N in excess to the unfertilized control from stabilized fertilizer products applied to native and limed Arborfield soil in a factorial design with fertilizer product and soil pH as the main factors.

Factor	Degrees of freedom	Sum of squares	Mean squares	*F*-value	*P*-value
Fertilizer	4	14.9	3.72	4.37	**0.01**
pH	1	19.5	19.5	22.9	**< 0.001**
Fertilizer × pH	4	5.17	1.29	1.52	0.23
Residuals	20	17.0	0.85		

Table A.14. Residual NH_4^+-N and NO_3^--N in excess to the unfertilized control from stabilized fertilizer products applied to Arborfield soil at thee different soil moisture levels. Different lowercase letters indicate significant differences between means groups at $P < 0.05$.

		Fertilizer product‡					
GSWC†		**U**	**UI-1**	**UI-2**	**NI**	**DI**	**Mean**
		NO_3^--N (mg)					
0.15	50%	13.42 ± 2.28	12.0 ± 2.25	11.0 ± 1.32	7.49 ± 2.31	5.75 ± 0.33	9.93 b
0.23	75%	19.85 ± 10.57	20.32 ± 15.13	20.01 ± 9.68	5.28 ± 8.13	6.99 ± 9.91	14.5 ab
0.31	100%	20.3 ± 1.49	18.93 ± 1.12	20.94 ± 1.82	15.31 ± 3.68	14.0 ± 0.95	17.9 a
Mean		17.9	17.1	17.3	9.36	8.91	
		NH_4^+-N (mg)					
0.15	50%	4.43 ± 1.19	4.69 ± 0.94	5.42 ± 1.87	8.33 ± 0.50	8.55 ± 0.66	6.29 a
0.23	75%	2.15 ± 1.24	1.57 ± 0.92	2.44 ± 2.01	4.23 ± 0.39	6.92 ± 1.42	3.46 b
0.31	100%	-0.7 ± 0.51	-0.26 ± 0.27	0.06 ± 0.39	5.11 ± 0.01	5.43 ± 1.15	1.93 b
Mean		1.97 b	2.00 b	2.64 b	5.89 a	6.97 a	

† Gravimetric soil water content, expressed as either g per g soil or as a percentage of field capacity.

‡ U = untreated urea, UI-1 = urease inhibitor (NBTPT), UI-2 = urease inhibitor (2-NPT), NI = nitrification inhibitor (DCD + TZ), DI = double inhibitor (NBTPT + DCD).

Table A.15. ANOVA table for residual NO_3^--N in excess to the unfertilized control from stabilized fertilizer products applied to Arborfield soil in a factorial design with fertilizer product and soil moisture content as the main factors.

Factor	**Degrees of freedom**	**Sum of squares**	**Mean squares**	***F*-value**	***P*-value**
Fertilizer	4	745.1	186.3	3.83	**0.011**
Moisture	1	78.3	78.3	1.61	0.21
Fertilizer × Moisture	4	166.1	41.5	0.85	0.50
Residuals	35	1704.0	48.7		

Table A.16. ANOVA table for residual NH_4^+-N in excess to the unfertilized control from stabilized fertilizer products applied to Arborfield soil in a factorial design with fertilizer product and soil moisture content as the main factors.

Factor	Degrees of freedom	Sum of squares	Mean squares	*F*-value	*P*-value
Fertilizer	4	200.7	50.2	11.2	**< 0.001**
Moisture	1	34.0	34.0	7.62	**0.009**
Fertilizer × Moisture	4	6.62	1.65	0.37	0.83
Residuals	35	156.2	4.5		

Table A.17. Residual NH_4^+-N and NO_3^--N in excess to the unfertilized control from stabilized fertilizer products applied to Arborfield soil at thee different soil temperatures. Different lowercase letters indicate significant differences between means groups at $P < 0.05$.

	Fertilizer product†					
Temperature	**U**	**UI-1**	**UI-2**	**NI**	**DI**	
	NO_3^--N (mg)					
5	-2.05 ± 3.88	3.36 ± 1.68	1.56 ± 3.42	2.94 ± 2.09	-0.55 ± 4.93	1.05 b
15	10.71 ± 3.10	17.80 ± 0.85	10.05 ± 5.58	7.84 ± 2.50	3.80 ± 1.73	10.0 a
26	19.85 ± 10.57	20.32 ± 15.13	20.01 ± 9.68	5.28 ± 8.13	6.99 ± 9.91	14.5 a
Mean	9.05	13.82	10.54	5.36	3.41	
	NH_4^+-N (mg)					
5	15.77 ± 0.08	15.79 ± 1.41	16.42 ± 1.31	18.58 ± 1.22	15.44 ± 2.03	16.40 a
15	4.40 ± 1.27	3.24 ± 0.65	4.23 ± 1.14	12.01 ± 1.79	10.88 ± 1.38	6.95 b
26	2.15 ± 1.24	1.57 ± 0.92	2.44 ± 2.01	4.23 ± 0.39	6.92 ± 1.42	3.46 b
Mean	7.44	6.87	7.70	11.62	11.08	

† U = untreated urea, UI-1 = urease inhibitor (NBTPT), UI-2 = urease inhibitor (2-NPT), NI = nitrification inhibitor (DCD + TZ), DI = double inhibitor (NBTPT + DCD).

Table A.18. ANOVA table for residual NO_3^--N in excess to the unfertilized control from stabilized fertilizer products applied to Arborfield soil in a factorial design with fertilizer product and soil temperature as the main factors.

Factor	Degrees of freedom	Sum of squares	Mean squares	*F*-value	*P*-value
Fertilizer	4	623.7	155.9	2.21	0.09
Temperature	1	535.2	535.2	7.58	**0.009**
Fertilizer × Temperature	4	273.5	68.4	0.97	0.44
Residuals	35	2470.4	70.6		

Table A.19. ANOVA table for residual NH_4^+-N in excess to the unfertilized control from stabilized fertilizer products applied to Arborfield soil in a factorial design with fertilizer product and soil temperature as the main factors.

Factor	Degrees of freedom	Sum of squares	Mean squares	*F*-value	*P*-value
Fertilizer	4	178.9	44.7	1.50	0.22
Temperature	1	425.3	425.3	14.24	**< 0.001**
Fertilizer × Temperature	4	27.5	6.9	0.23	0.91
Residuals	35	1045.2	29.9		

A.3 Calculation of non-metric multidimensional scaling of cumulative emission profiles

To summarize the performance of stabilized fertilizers across a range of environmental conditions using non-metric multidimensional scaling, the cumulative emissions of each treatment across all field measurement periods or bench-scale experiments were added to a dataframe and considered the individual "emission profile". An example for the emission profile is shown in Table A.20. Each row within the table represents the cumulative emission profile of the respective fertilizer treatment replicate. Samples were then *log(1 + x)* transformed and the non-metric multidimensional scaling of transformed data calculated using the Bray-Curtis dissimilarity index according to Clarke and Warwick (1994). Stress levels for the ordinations (Figs. 6.1, 6.2, and 6.3)

were low (i.e., 8.65, 0.16, and 4.96, respectively) and thus reflect the good agreement between rank dissimilarities and the distance in ordination space.

Table A.20. Cumulative emission profiles of each treatment replicate across the eight bench-scale volatilization experiments in chapter 5.

Treatment†	Replicate	Exp. 1	Exp. 2	Exp. 3	Exp. 4	Exp. 5	Exp. 6	Exp. 7	Exp. 8
		mg N							
C	1	0.04	0.01	0.04	0.03	0.02	0.00	0.02	0.02
C	2	0.06	0.03	0.01	0.08	0.01	0.02	0.02	0.04
C	3	0.11	0.05	0.03	0.26	0.07	0.04	0.05	0.11
U	1	0.97	0.78	3.16	2.06	0.91	2.37	1.49	1.67
U	2	0.86	0.86	3.69	2.14	0.85	2.08	2.17	1.61
U	3	0.95	1.03	5.76	3.62	1.52	1.55	0.83	2.01
UI-1	1	0.28	0.57	2.50	3.67	1.37	1.44	1.72	1.57
UI-1	2	0.40	0.55	3.16	3.20	1.15	2.46	1.43	1.64
UI-1	3	0.40	0.89	2.89	3.99	1.56	1.75	0.84	1.47
UI-2	1	0.49	0.64	1.78	2.77	1.15	1.12	0.40	0.76
UI-2	2	0.89	0.52	1.69	1.38	1.36	1.21	1.11	1.48
UI-2	3	0.86	0.66	0.82	3.05	0.96	0.82	0.84	1.40
NI	1	1.34	1.12	2.71	2.06	1.27	2.56	1.44	1.55
NI	2	1.07	1.04	4.69	2.71	2.36	1.87	1.68	3.32
NI	3	1.46	1.83	3.69	3.71	2.12	1.66	1.32	1.85
DI	1	0.51	0.38	1.24	0.57	0.70	1.28	0.03	0.37
DI	2	0.57	0.37	1.10	0.90	0.45	0.35	0.13	0.71
DI	3	0.25	0.39	1.96	0.72	0.35	0.56	0.20	0.73

† C = unfertilized control, U = untreated urea, UI-1 = urease inhibitor (NBTPT), UI-2 = urease inhibitor (2-NPT), NI = nitrification inhibitor (DCD + TZ), DI = double inhibitor (NBTPT + DCD). Each treatment replicate is assigned the profile of its cumulative emissions (i.e., each row) throughout various soil conditions (i.e., Experiment 1 to 8 of the bench-scale experiments in Chapter 5, see Table 5.3). Using non-metric multidimensional scaling, these profiles can be ranked according to their similarity.